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A Method for Continuous Measurement of the Carbon Dioxide Tension on the Cerebral Cortex

By

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Abstract

Siesjö, B. K. *A method for continuous measurement of the carbon dioxide tension on the cerebral cortex.* Acta physiol. scand. 1961. *51.* 297—313. — A method has been developed for direct, continuous measurement of the pCO_2 on the surface of the cerebral cortex, using a modification of the tissue pCO_2 electrode earlier described (Hertz and Siesjö 1959). The modification was made for the purpose of increasing the stability and accuracy of the measurements. The drift of the electrode was thereby reduced to less than 1 mV in five hours and the accuracy was increased to about $\pm$ 1.5 per cent. A reduction of the concavity of the pH glass membrane limited the response time of the pCO_2 electrode to 25—30 sec following a twofold increase in pCO_2. A description is given of the procedures for calibrating, suspending and thermostatting the electrode.

The validity and reproducibility of the pCO_2 values obtained when the electrode was applied to the cortical surface were investigated in cat experiments during respiratory and circulatory steady states. It was found that within a relatively wide range the pCO_2 was independent of the pressure exerted by the electrode on the cortical surface. Provided that the tissue did not present any swelling and that the tissue surface was not damaged or allowed to dry, the pCO_2 varied very little (coefficient of variation 1.5 per cent) when the electrode was repeatedly applied to the same or to different cortical areas. The influence of superficial cortical vessels was confined to the larger veins (diameter 0.5—1 mm), across which slightly higher (1—5 mm Hg) values were recorded. The findings are discussed in relation to the applicability of the method in studies of the acid-base metabolism of the tissue.

The importance of carbon dioxide for functional and circulatory processes in the brain (*cf.* survey by MEVES 1958) has mainly been studied indirectly, *i. e.* through the physiological effects induced by changes in the carbon dioxide content of the inspired air and the blood. The only direct study of the carbon dioxide exchange of the tissue has dealt with single analyses of the total carbon dioxide content of cortical tissue samples (BRODIE and WOODBURY 1958, NICHOLS 1958). In these studies, however, it was possible to draw quantitative conclusions as to the acid-base metabolism of the tissue only after assumptions had been made with regard to the pCO_2 of the tissue, the solubility coefficient of carbon dioxide, and other factors. In no instance has a continuous measurement of any aspect of the carbon dioxide metabolism of the tissue proper been possible.

The development of a pCO_2 electrode for tissue measurements (HERTZ and SIESJÖ 1959), however, provides certain possibilities in this respect (*cf.* INGVAR, SIESJÖ and HERTZ 1959). The original electrode had the advantage of a suitable geometrical form, sensitivity and fastness in response. Yet a troublesome drift was a considerable drawback. Before the electrode could be made into a good instrument for quantitative investigations of the pCO_2 of the tissue, its stability had to be improved as well as the accuracy of the measurements. In addition, factors which influence measurements on the surface of the tissue had to be analyzed.

The theoretical background for the measurement of the tissue pCO_2 is the diffusibility of carbon dioxide and the potentiometric nature of the electrode principle (STOW, BAER and RANDALL 1957). It follows that the electrode, if properly applied, should attain gaseous equilibrium with the tissue when placed on the surface of it. The value of pCO_2 obtained on the arachnoidal membrane of a given cortical area cannot, however, be directly used as an expression of the pCO_2 of the tissue proper. Thus, gradients of pCO_2 must exist on the surface of the brain in relation to superficial arteries and veins. The extent to which these vessels influence the pCO_2 of different cortical spots obviously limits the importance of any quantitative conclusions if these are based on measurements on the surface. If so, they are not valid for the cortical grey matter as a whole. Differences in pCO_2 between different cortical areas also tend to impair the value of such conclusions.

The aim of the present work is twofold. In the first place it describes suitable modifications of the tissue pCO_2 electrode so as to increase its stability as well as the accuracy of the measurements. Secondly, it is intended as a methodological study of the problems encountered in the course of measurements on the surface of the feline cerebral cortex. The primary problem here was to investigate the optimal conditions for obtaining a minimum variation of pCO_2 at a given cortical locus in a respiratory and circulatory steady state. When such conditions were felt to be present, the variations of pCO_2 encountered within the same or between different cortical areas were studied. The

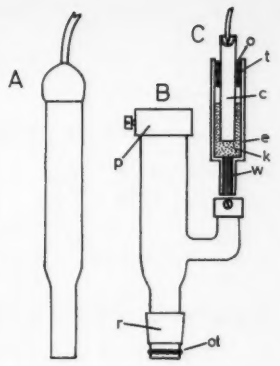

Fig. 1. The pCO_2 electrode unassembled. Only the calomel reference electrode shown in cross-section. *A:* shielded pH electrode. *B:* glass housing with cover and screw of Perspex (p) for the fastening of the pH electrode; rubber tubing (r) and o-ring (ot) to secure the Teflon membrane. *C:* calomel reference electrode with inner core (c); Perspex housing (e); o-rings (o) to keep the core in the housing, held apart by a Perspex tubing (t); cotton wick (w) and potassium chloride (k).

relation between the pCO_2 thus obtained and an average tissue pCO_2 determined for the tissue proper will be dealt with elsewhere (GLEICHMANN *et al.* 1961).

Material and methods

A. *The pCO_2 electrode*

It seemed probable that the drift of the original electrode was, at least in part, due to base dissolved from the glass. It was therefore necessary to find a kind of glass with especially low alkali solubility[1]. At the same time electrodes with more or less pronounced concavities of the glass membranes were tested. Another procedure tried for stabilization was the intercalation of a special brand of paper (GIZEH-paper, SCHOELLER and HOESCH, Gernsbach, Muretal) between the pH electrode and the Teflon membrane, as used in a newly devised electrode for blood pCO_2 measurements (GLEICHMANN and LÜBBERS 1960).

The miniature *calomel reference electrode* earlier used (HERTZ and SIESJÖ 1959) had a tendency to dry and could add to the drift of the pCO_2 electrode by means of changing diffusion potentials. An electrode with a greater potassium chloride capacity was therefore used instead[2] (Fig. 1). Contact between the potassium chloride solution (k) and the outer bicarbonate solution was made through a cotton wick (w). In assembling the reference electrode a small piece of cotton (w), soaked in the bicarbonate solution used, was packed into the lower end of the Perspex tube (e). This was then half filled with saturated potassium chloride (k). The core (c) with its o-rings (o) was introduced from above and held in position by the tight fitting of the o-rings (o). Excess potassium chloride was allowed to escape through small openings (not shown in the figure) in the upper part of the Perspex tube (e). If silicone grease was applied to the Perspex around these openings, there was no need to refill the potassium chloride for several days.

As Perspex was found to dissolve appreciable amounts of carbon dioxide, *the electrode*

[1] The generous help given by the firms INGOLD (Frankfurt am Main) and RADIOMETER (Copenhagen) is gratefully acknowledged.

[2] The inner core of the calomel reference electrode was kindly supplied by RADIOMETER.

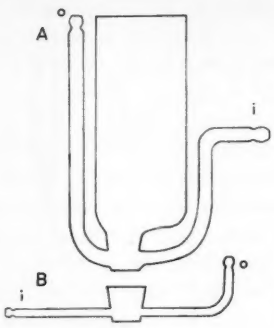

Fig. 2. Calibration chamber in glass with inlet (i) and outlet (o). *A:* chamber for thermostatting the whole pCO_2 electrode. *B:* chamber for thermostatting the lower end of the electrode.

housing (B) was made of glass with a cover of Perspex (p) for the fixation of the pH electrode (A) (Fig. 1). The lower end of the housing had a groove for an o-ring (ot), which held the Teflon membrane (6 or 12 micron) in position. A piece of rubber tubing (r) around the lower end of the housing served to adjust the electrode in the calibration chamber. As the pH electrodes later supplied (INGOLD, Frankfurt am Main) were shielded, screening of the electrode housing was omitted.

In *assembling the electrode* the following procedure was followed: a sheet of the Teflon membrane (about 4 cm in diameter) was placed upon the o-ring and the lower end of the electrode was pressed against the o-ring until it slid onto the housing. The o-ring was then transferred into the groove of the glass housing and the excess Teflon membrane above the o-ring was wrapped around the rubber tubing, above which it was secured with a rubber band. If instead the Teflon membrane was cut immediately above the o-ring, there might be electrical leaks in the course of the measurements on the tissue. Next, the housing was filled with the sodium bicarbonate solution (0.001 or 0.0001 N), and it was carefully seen to it that no air bubbles stuck to the Teflon membrane. At this stage the Teflon was tested for holes by gentle blowing at the end of the housing with the side branch closed. If there were any leaks in the membrane — even small ones — a small liquid drop was formed by the blowing. The pH electrode was then introduced into the housing and secured by means of the Perspex screw when the end of the electrode exerted a slight pressure on the Teflon. Finally the calomel reference electrode was introduced into the side branch of the electrode housing.

If GIZEH-paper was inserted between the pH electrode and the Teflon membrane, a circular piece of the paper (diameter equalling the outer diameter of the electrode housing) was cut with a cork bore, thoroughly moistened with the bicarbonate solution and placed on the Teflon membrane so that it covered the o-ring. The same procedure as described above was then followed.

The instrument used for the measurements was a pH meter (pH meter 22, RADIOMETER, Copenhagen), on which differences of 0.5 mV could be read conveniently. The ground lead of the shielded wire of the pH electrode was connected to a good ground, which also received a lead from the water bath (see below). The electrical stability was increased if some sodium chloride (about 1 per cent) was added to the water bath (*cf.* ASTRUP 1959).

Calibration of the pCO_2 electrode was performed with streaming gas, which was preheated and saturated with water vapour in a water bath of constant temperature ($\pm$ 0.1° C.). The gas slowly passed the calibration chamber which received the elec-

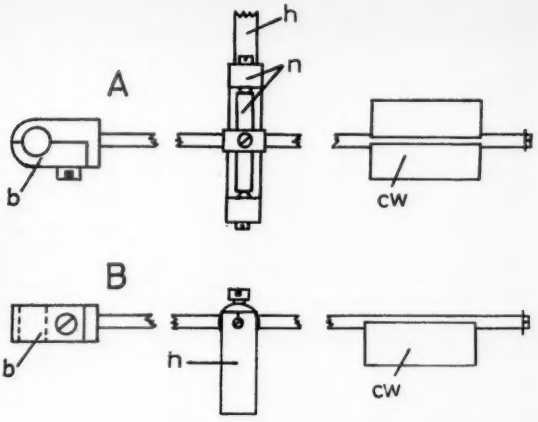

Fig. 3. Electrode holder, viewed from above (A) and from the side (B) with needle bearing (n), bakelite clamp (b), counter-weight (cw) and holder (h). The clamp is sized to fit the side branch of the electrode housing.

trode (Fig. 2 A). Humidifiers, all rubber tubes, and the inlet to the calibration chamber were kept below the water surface. Unless these precautions were taken the value obtained for a given gas concentration varied with the speed of the gas stream. Owing to the light sensitivity of the inner reference electrode of the pH electrode, the latter was protected against direct sunlight during calibration.

The type of calibration chamber shown in Fig. 2 A made it possible to thermostat the whole pCO_2 electrode in the water bath. To avoid convection in the air enclosed in the chamber, cotton wool was loosely packed in the upper end around the electrode.

In *thermostatting* the pCO_2 electrode for tissue measurements, any measure which appreciably increased the weight of the electrode had to be avoided. The most satisfactory of the methods tried was the use of a Perspex box around the head of the animal with holes for electrical leads, respiratory tubes, etc. Heated air was blown into the box by a hair drier placed in a funnel at the top of the box. The air escaped through the holes. The temperature of the circulated air was adjusted to that of the cortical surface by a change in the voltage across the heater of the drier. For this purpose the leads to the heat generating resistor were isolated and connected to a variable autotransformer. A syringe in the wall of the box and a polyethylene catheter with its end lodged near the craniostomy made it possible to irrigate the surface of the brain with Ringer's solution at body temperature.

A simpler but less accurate way of obtaining temperature control was also tried. As before, the temperature of the cortical surface was carefully measured with an electrothermometer based upon the thermoelement principle (ELEKTROLABORATORIET, Copenhagen) and the temperature of the water bath was adjusted accordingly. For calibration the chamber shown in Fig. 2 B was used. It was immersed into the water to a depth where the Teflon membrane of the pCO_2 electrode was just below the water surface. The procedure ensured fairly identical temperature conditions for the pH glass and the enclosed bicarbonate solution during calibration and measurement ($\pm$ 0.3° C.). In both situations the calomel reference electrode was exposed to room air, the temperature of which could be kept reasonably constant ($\pm$ 0.5° C.) by means of an air thermostat (BRAUN, Frankfurt am Main).

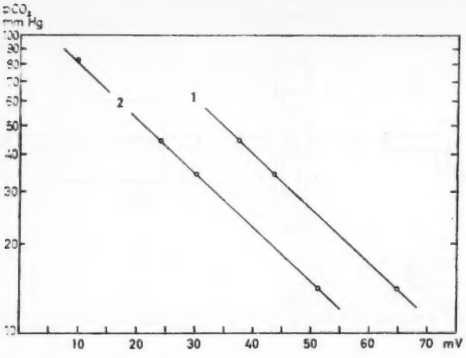

Fig. 4. Calibration curve obtained on the day of assembling the pCO_2 electrode (1) and after seven days (2). 37° C. and 0.0001 N $NaHCO_3$. Note parallel displacement of calibration curve and deviation from linearity at about 80 mm Hg.

For the measurements on the surface of the cerebral cortex a mechanical *balancing device* was constructed, which minimized the pressure of the electrode on the tissue. The side branch of the electrode housing was fastened at one end of a lever, which was suspended on a needle bearing (Fig. 3). The weight of the electrode (30—35 grams) was balanced by a movable counter-weight. In this way the electrode readily followed small movements of the tissue synchronous with *e. g.* the respiration.

B. *The tissue measurements*

Experiments with observation of tissue pCO_2 were made on 24 cats. All animals were anesthetized with Nembutal (initial dose 40 mg per kg body weight i.p.). In cases where artificial respiration was employed, a curarizing agent (Flaxedil) was administered. The femoral vein was exposed and cannulated for injections and the femoral artery was treated in the same way for recording the blood pressure by means of an electro-manometer (ELEMA, Stockholm). The scull bone was exposed through resection of the temporal muscles and one or more holes were drilled in the bone, one of which usually over the Suprasylvian gyrus. The dura was carefully split and the flaps were folded back over the bony edges. The exposed cortical surface was protected from drying by intermittent irrigation with Ringer's solution.

In most experiments the electrode was assembled with 0.001 N $NaHCO_3$ and with interposed GIZEH-paper. The electrode had a weight of about 35 g. The lower end of the electrode housing had a diameter of 8.5 mm. Temperature control was obtained by calibration at the same temperature as that of the cerebral cortex, and the larger part of the electrode was exposed to ambient air during both calibration and measurement. When the dura had been opened the electrode was mounted in its holder, which was then placed close to the craniostomy so that the Teflon membrane of the electrode was parallel to the surface of one of the gyri. The counter-weight of the electrode holder was then adjusted until the electrode was almost outbalanced and lowered so that the electrode touched the tissue surface. Extreme care was taken so that the electrode readily followed the respiratory movements of the tissue without causing any dimpling of the tissue surface. The thermojunction of the electro-thermometer was lodged beneath the dura at the border of the craniostomy. The temperature of the water bath used for calibration was then adjusted to that of the cortical surface so as to permit calibration immediately after a series of measurements.

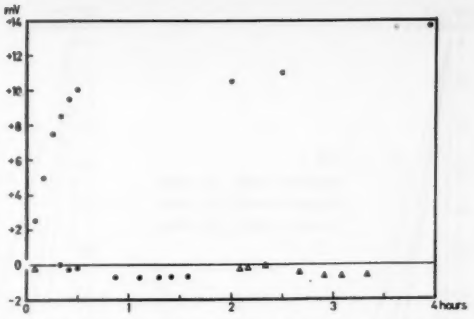

Fig. 5. Stability of the pCO_2 electrode assembled with different pH electrodes, as tested with a continuous gas flow and at a fixed temperature (37.0° C.). The long intervals in the individual records denote periods when the electrode was applied to the cortical surface in cat experiments. All pH electrodes were made from glass of low alkali solubility (see methods), either with an ordinary smooth glass surface, unstabilized (open circles) and stabilized with GIZEH-paper (triangles), or with a porous surface (closed circles). Note fast drift towards alkalinity with the smooth type of pH electrode and irregularities in the alkaline displacement following application to the tissue (pressure sensitivity).

Results

A. *Electrode characteristics*

The *sensitivity* of a pCO_2 electrode, which has been defined as $\frac{\triangle \text{ pH}}{\triangle \log pCO_2}$ (SEVERINGHAUS and BRADLEY 1958), is influenced by the bicarbonate concentration and by the buffering capacity of any material used to keep a constant layer of reference solution between the glass membrane and the Teflon membrane. The actual mV sensitivity for a given change in pCO_2 is, however, also dependent upon the sensitivity of the pH electrode. Thus there was a danger of substantially reduced sensitivity in the case of the tissue pCO_2 electrode, where special pH electrodes were used in connection with dilute bicarbonate solutions and where an interposed membrane was sometimes used for stabilization. It was found, however, that the actual sensitivity of the electrode (1 pH unit = 61.5 mV at 37° C.) assembled with 0.0001 N $NaHCO_3$ and with interposed GIZEH-paper was 0.88—0.90 (Fig. 4). At higher bicarbonate concentrations and without the GIZEH-paper the sensitivity increased correspondingly (*cf.* SEVERINGHAUS and BRADLEY 1958, GLEICHMANN and LÜBBERS 1960).

Since the sensitivity changes with hydrogen ion concentration (SEVERINGHAUS and BRADLEY 1958), the calibration curve could possibly be non-linear within physiological carbon dioxide concentrations. With the gas mixtures used in the present investigation (2 to 12 per cent carbon dioxide) no deviation from *linearity* could be detected using 0.001 N $NaHCO_3$ solution. With the 0.0001 N $NaHCO_3$ solution, however, a small deviation from linearity could be seen at about 80 mm Hg (*cf.* Fig. 4). The dependence of the sensitivity upon the hydrogen ion concentration was noticeable when pCO_2 electrodes with an alkali drift were used. Here the sensitivity was not constant but in-

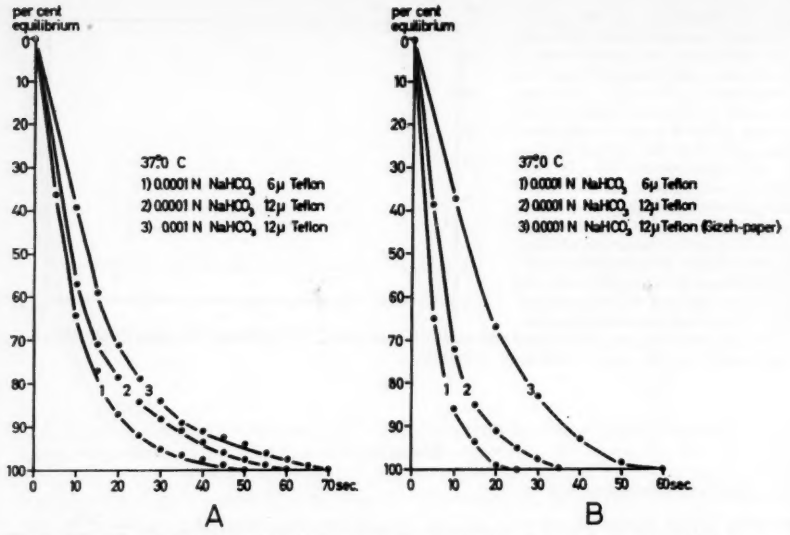

Fig. 6. Response time of the pCO_2 electrode obtained after a twofold increase in pCO_2 (usually 16 to 35 mm Hg). The curves were obtained with the porous type of pH electrode, those in the right part (B) with a relatively flat glass membrane.

creased with the drift towards alkalinity, *i. e.* the slope of the calibration curve changed with time.

The *stability* of the pCO_2 electrode is influenced by the characteristics of the pH electrode and by the stability of the calomel reference electrode. The latter was tested in a 0.001 N $NaHCO_3$ solution against a commercial calomel reference electrode with an open liquid junction (K 100, RADIOMETER, Copenhagen). If the cotton wick of the reference electrode was previously equilibrated with the bicarbonate solution, the potential difference between the electrodes did not exceed 2 mV, any change usually being less than 1 mV in 12 hours.

The use of pH electrodes with glass membranes of low alkali solubility did not prevent the drift of the pCO_2 electrode previously experienced. As before, the electrode displayed an initially fast, but later slower drift towards alkalinity (Fig. 5, open circles). If, however, GIZEH-paper was used there was only an insignificant drift towards the acid side. This latter drift did not usually exceed 1 mV in 5 hours and was often less than 1 mV in 10 hours (Fig. 5, triangles). It did not matter if the GIZEH-paper was allowed to cover the pH glass surface in the form of a sheet, or if it was inserted between the Teflon membrane and the edge of the pH electrode in the form of a ring. The acid drift of the pCO_2 electrode caused a slow, parallel displacement of the calibration

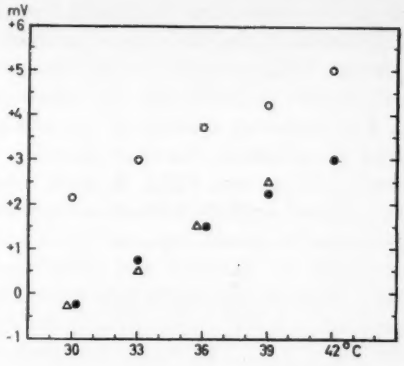

Fig. 7. Temperature coefficient of the pCO_2 electrode as tested with a continuous gas flow (6.23 per cent carbon dioxide). 0.0001 N $NaHCO_3$ and GIZEH-paper. No correction made for the change in the water saturation of the gas. The values denoted by open circles and closed circles (different experiments) were obtained during heating, those denoted by triangles during cooling in one of the above experiments (represented by the closed circles).

curve. This displacement, followed for periods of up to 7 days, was, however, so small that no change in sensitivity could be detected (Fig. 4).

One type of pH electrode tested (INGOLD, Frankfurt am Main) made the pCO_2 electrode sufficiently stable without the help of an inserted paper (Fig. 5, closed circles). It was found that the surface of the glass membrane of this type of pH electrode was slightly porous, with a milky appearance. A change of this type extending over only a part of the glass membrane and the edge of the electrode was sufficient to render the assembled pCO_2 electrode stable.

The *response time* of a pCO_2 electrode depends, among other things, upon the bicarbonate concentration and on the thickness of the gas permeable membrane (SEVERINGHAUS and BRADLEY 1958). The use of pH electrodes of the present type added another variable, the concavity of the glass membrane, in that a relatively flat membrane gave a faster pCO_2 electrode. Fig. 6 A shows the response time obtained after a twofold increase in pCO_2 using the above-mentioned porous type of pH electrode with different bicarbonate concentrations and membrane thicknesses. If a pH electrode with a very flat glass membrane was included, the result was a fast pCO_2 electrode. Thus, with 0.0001 N $NaHCO_3$ and 6 μ Teflon, 70 per cent equilibrium was reached after 6—7 seconds and 100 per cent after about 25 seconds (Fig. 6 B). In this case there was a slight drift towards alkalinity. Stabilization with GIZEH-paper prolonged the response time so that changes were obtained which equalled those resulting from the ordinary type of electrode.

The *sensitivity to pressure* of the pCO_2 electrode had to be taken into account when the electrode displayed an alkali drift. A sudden pressure on the Teflon membrane often gave a displacement of the calibration curve, mostly to the acid side. This effect appeared to be greater the more the drift had changed the original pH value (*cf.* Fig. 5). Accordingly, small effects or no effects

at all were seen when the present type of pCO_2 electrode was used. Repeated calibrations during the measurements on the brain also showed that the steady pressure of the electrode on the tissue even during large respiratory movements or vigorous pulsations did not influence the calibration curve.

The *temperature sensitivity* of the pCO_2 electrode was investigated with the type of calibration chamber shown in Fig. 2 A and with a continuous gas flow (6.23 per cent CO_2). A stable value after a change in temperature was not attained until after about one hour and the time of observation was usually prolonged to about one and a half hour at each temperature. Using 0.001 or 0.0001 N $NaHCO_3$ and GIZEH-paper, small temperature effects were seen. Thus, in the region between 30° and 42° C. a change of 4° altered the reading by 1 mV, in the alkaline direction during heating, in the acid during cooling (Fig. 7). This means that around 37° C. and at a pCO_2 of 45 mm Hg, a change in temperature of one degree changed the pCO_2 value obtained by 0.5 mm Hg.

The *accuracy* of the measurements as tested with repeated calibrations with the same gas mixture or repeated applications of the electrode to the calibration chamber was calculated according to the formula

$$\sigma^2 = \frac{ij\,(X_{ij} - X_{.j})^2}{N - j}$$

σ = Standard deviation
X_{ij} = measured value in mm Hg
$X_{.j}$ = mean of the j:th group
N = number of observations

All the groups (j) were supposed to belong to the same population. The results were transformed into a coefficient of variation (V) by applying the formula

$$V = \frac{100\,\sigma}{\text{mean}}$$

When calculated from 37 measurements in 8 groups, including measurements with the less accurate way of temperature control as well as measurements with intervals of a couple of hours, a coefficient of variation of 1.6 per cent was obtained.

B. *The tissue measurements*

The absolute pCO_2 as measured on the surface of the cerebral cortex of the anesthetized cat is influenced by several factors, the most important of which being changes in the pulmonary ventilation. As the chief aim of the tissue measurements in the present investigation was to determine the reproducibility of a pCO_2 value obtained in a given or in different cortical fields, the experiments were carried out in a steady state, *i. e.* the animals were usually ventilated artificially and kept in a moderate or deep barbiturate anesthesia. To exclude the possibility that the artificial respiration or the deep anesthesia was responsible for a stability that did not occur otherwise, a few

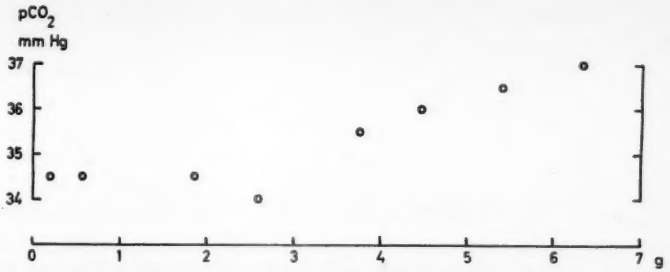

Fig. 8. Cat, Nembutal anesthesia and artificial respiration. Influence of the mechanical pressure of the electrode upon the value of pCO_2 obtained on repeated application of the electrode to the same area (middle suprasylvian gyrus). Each circle denotes pCO_2 at equilibrium (judged as constant reading for two minutes). The electrode pressure was increased by a change in position of the counterweight of the electrode holder. Diameter of the lower end of the pCO_2 electrode 8.5 mm. At the pressure where the pCO_2 started to increase (about 3 grams) the tissue surface was dimpled 0.5—1 mm.

experiments were carried out with lightly anesthetized, spontaneously breathing preparations.

After application of the electrode to the exposed arachnoidal surface of an arbitrarily chosen cortical area, an equilibrium was usually obtained after 3—6 min. If a steady state prevailed with regard to respiration, blood pressure and anesthesia, and if temperature fluctuations were avoided, the pCO_2 thus obtained varied very little for periods of up to hours, and in many cases the variations during that time did not exceed a range of 2 mm Hg.

In the majority of the experiments the electrode was applied to a cortical area, which was exposed through a drilled hole of slightly greater diameter than that of the electrode. Since it was possible that carbon dioxide could be lost by diffusion from the surface, which might result in a false equilibrium, vaseline was in a few experiments applied around the electrode in the drilled hole. This, however, was found to cause no change in the pCO_2 value previously recorded. Nor was there any increase in pCO_2 if a gas mixture containing carbon dioxide at the same partial pressure as that measured was circulated around the lower end of the electrode.

The stability of the value of recorded pCO_2 depended upon a good contact between the electrode and the tissue. If the pressure was too slight when the electrode was applied so that it intermittently lost its contact with the tissue surface, a lower and unstable value was obtained. The electrode could easily, however, be applied in such a way that it remained in contact with the tissue even during relatively large respiratory excursions without causing any dimpling of the tissue surface. If, then, an increased pressure was applied to the tissue by a change in the position of the counter-weight, the value obtained did not change significantly until the tissue surface was dimpled to a depth of about 1 mm. In these cases slightly higher values were recorded although the cali-

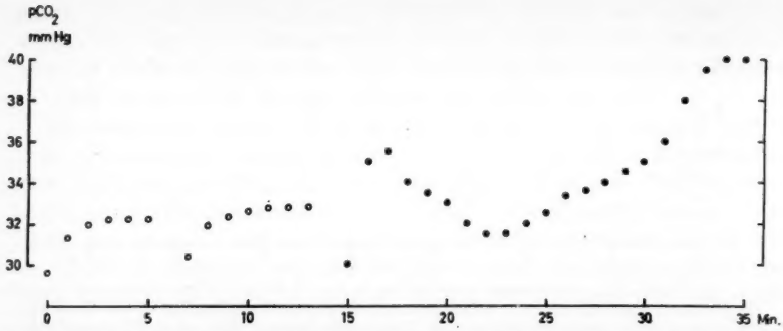

Fig. 9. Cat, Nembutal anesthesia and artificial respiration. Effect of vessel injury at the cortical locus, where the pCO_2 was repeatedly measured (middle suprasylvian gyrus). The open circles denote readings at 1 minute intervals in the course of two applications of the electrode. After 13 minutes three small superficial vessels were torn with a surgical needle, causing limited subarachnoidal bleedings. Note unstable and rising pCO_2 (closed circles) after the vessel injury.

bration curve of the electrode was not affected. In one such experiment the pressure exerted by the electrode was measured in grams. It was then found that the pressure amounted to 3—4 g at the point where the pCO_2 value began to increase (Fig. 8).

The electrode holder made it possible to lift the electrode from the tissue surface and, after allowing time for partial equilibrium with air, to reapply it to the same cortical locus, or to different loci within the same area. If this was done repeatedly the pCO_2 value could usually be reproduced within a range of 1 mm Hg. The reproducibility thus tested did not change if the liquid on the cortical surface was carefully sucked away and if the surface was then irrigated with *e. g.* Ringer's solution, or if the tissue surface became polluted by blood from leaking bone vessels. This reproducibility was obtained on condition that there was no increased pressure in the brain with bulging in the bore hole and that the cortical surface was not allowed to dry. In the latter case, due to exposure to air during longlasting experiments, a decreased reproducibility was often obtained as well as an increased susceptibility to the pressure of the electrode. A third factor of importance for the constancy of the pCO_2 value obtained was an intact pial circulation. Thus, if the superficial pial vessels under the electrode were punctured or torn, unstable values resulted (Fig. 9).

A large one-sided craniotomy with exposure of parts of two adjacent gyri made it possible to apply the electrode to a number of different cortical areas, avoiding direct application over the largest superficial vessels. A respiratory steady state could usually be upheld for periods of up to an hour, allowing 5 to 9 measurements in a group. The variations in the pCO_2 values between the different seats of application were not greater than those found on repeated

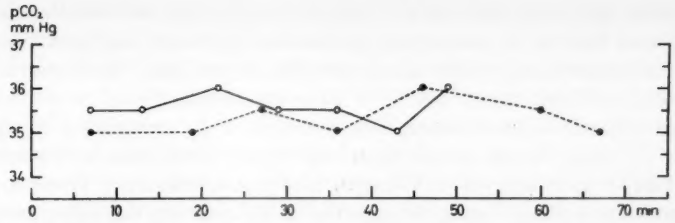

Fig. 10. Cat, Nembutal anesthesia, in one experiment with spontaneous (open circles), in the other with artificial respiration (closed circles). Reproducibility of value of pCO_2 obtained on repeated application of the electrode to different cortical areas (Sylvian and suprasylvian gyrus). Constancy for two minutes judged as stable end value. In both experiments the values obtained varied within a range of 1 mm Hg.

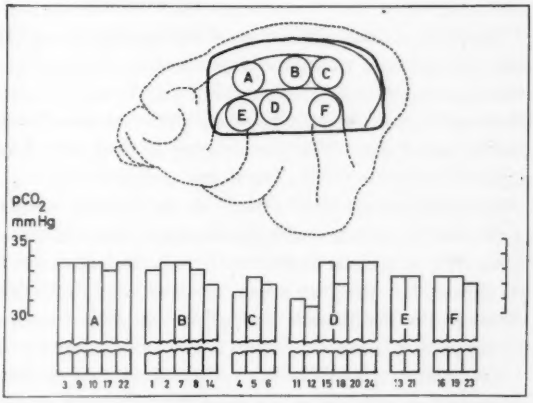

Fig. 11. Cat, Nembutal anesthesia and spontaneous respiration. Reproducibility of value of pCO_2 obtained on repeated application of the electrode to different cortical spots (A—F) of an animal, which was kept in superficial anesthesia. Constancy for two minutes judged as stable end value. The individual measurements (in chronological order) denoted at the bottom of the figure. Occasionally during the experiment the animal was seen to move slightly with associated polypnoe (before measurements 5, 13 and 19). When this occurred additional anesthesia (2—3 mg Nembutal per kilo body weight i.v.) was given. Note small variations in the pCO_2 values obtained (coefficient of variation 2.6 per cent) in spite of long observation time (about two and a half hour) and correlation between the lowest values and the observed polypnoe.

application of the electrode to the same area, *i. e.* the values usually varied within a range of 1 mm Hg, irrespective of whether the animal was breathing spontaneously or was artificially ventilated (Fig. 10).

If all measurements with 5 or more determinations in a group (same or different spots) were put together and all experiments with observed changes in respiration and blood pressure during the time of measurement were discarded, 7 groups with 42 measurements were obtained. Statistical treatment based upon the assumption that all measurements belonged to the same population (see above) gave a coefficient of variation of 1.5 per cent.

Factors which tended to decrease the reproducibility of the pCO_2 values obtained during a steady state were superficial anesthesia during spontaneous

respiration and prolongation of the time of measurement, an example of which is shown in Fig. 11. It is seen that in this case there was a greater variation in the values obtained (coefficient of variation 2.6 per cent). The lowest values, however, correlated pretty well with observed polypnoe.

The influence of pial vessels was investigated by comparing the pCO_2 values obtained directly across superficial arteries and veins with those obtained in an area without such vessels. The pial arteries were found to have no significant influence upon the reproducibility, whereas the values obtained over large veins (diameter 0.5—1 mm) was 1—5 mm higher than elsewhere on the cortex. Although the pCO_2 as obtained over different veins varied directly with the diameter of the vessel, the values measured over a given vein did not seem to vary more than in an area void of large veins.

Discussion

The pCO_2 electrode. The way of assembling the pCO_2 electrode must depend upon the type of experiment performed. Thus, the use of GIZEH-paper has the advantage that pH electrodes with ordinary smooth glass surfaces can be used and that the concavity of the pH glass membrane can be reduced without risking unstability. The porous type of electrode, however, is preferable when rapid changes in pCO_2 are to be recorded.

For ordinary use the 0.001 N $NaHCO_3$ solution is recommended on account of the better linearity at higher carbon dioxide tensions than with other solutions. When a short response time is desirable the 0.0001 N solution should be chosen. In the latter case the use of 6 μ Teflon is a further advantage although the mechanical strength of the 12 μ membrane makes it better suited for most situations.

Two things are difficult to explain: the cause of the reduced drift and the decreased sensitivity to pressure of the modified tissue pCO_2 electrode. The most probable explanation seems to be the following: In the electrode arrangement the lower end of the pH electrode exerts a slight pressure on the Teflon membrane. A small volume of bicarbonate solution between the Teflon and the pH glass membrane is thereby separated from the main bulk of the solution except for a narrow connection around the edge of the pH electrode. The pCO_2 electrode is sensitive to changes in the hydrogen ion concentration of the minor bicarbonate compartment. Such changes occur if carbon dioxide diffuses through the Teflon or if acid or basic substances are dissolved from the pH glass. It is known that base is dissolved from all kinds of pH glass (*cf.* KRATZ 1950), and this is thus the most probable explanation of the alkaline drift of the electrode. The present work has shown that there were two ways of avoiding the drift, either by using pH electrodes with a porous glass surface or by using stabilizing GIZEH-paper. This paper can be assumed to have a twofold effect, partly it acts as a buffer, partly — by virtue of its pores — it allows substances to diffuse into the main bulk of the solution. The latter

effect seems to be the most important one, since a stabilization was obtained also with the porous type of pH electrode. It can thus be assumed that both procedures used for stabilization allowed the free diffusion of dissolved base from the minor bicarbonate compartment into the major one. Probably this exchange did not occur when there was a firm contact between the smooth edge of a pH electrode and the Teflon.

It seems that the sensitivity of the original pCO_2 electrode to be the pressure applied to the Teflon membrane was due to variations in volume of the smaller bicarbonate compartment. Thus, an unequal distribution of base between the two compartments would be changed when pressure on the Teflon caused an exchange of solution between the compartments. Accordingly, an electrode in which any base dissolved from the pH electrode is more uniformly distributed in the whole bicarbonate solution will be more resistant to pressure.

The tissue measurements. The cerebral cortex must be assumed to be subject to the same physico-chemical laws with regard to solubility and hydration of carbon dioxide and dissociation of carbonic acid as other systems in the organism. It follows from these laws that carbon dioxide, being a gas, must exert a certain pressure at each point in the tissue and that this pressure has a certain local relation to the hydrogen ion concentration and the bicarbonate concentration. If the pCO_2 changes these last-mentioned variables change, and vice versa. Provided that the relations in the bicarbonate-carbonic acid buffer system of the tissue can be experimentally clarified, it is clear that the direct determination of one of the variables mentioned can give valuable information on the acid-base metabolism of the tissue proper.

Owing to the lack of proper methods, information on the carbon dioxide metabolism of the cerebral cortex is scanty and mostly drawn from indirect studies. The only variable which has been studied directly, is the total carbon dioxide content of samples of tissue removed from animals which had previously been allowed to breathe different concentrations of carbon dioxide (Nichols 1958, Thompson and Brown 1960). This method necessitated the decapitation of the animal for a single analysis. It was possible that the pCO_2 changed between the time of decapitation and the time of analysis. Besides, the pH and the bicarbonate concentration of the tissue could only be calculated (Brodie and Woodbury 1958) after a series of assumptions concerning the actual tissue pCO_2, the solubility coefficient for carbon dioxide, and other factors.

It is obvious that a uniform pCO_2 does not exist in the tissue where tension gradients are built up in the course of diffusion processes. It is convenient for practical reasons to express the different tensions of a gas in a tissue as an average tension (Thews 1953, Kety 1960). Accordingly, if useful in quantitative investigations, the pCO_2 value obtained by a tissue pCO_2 electrode should ideally either be identical with or have a constant relation to the average tension in the tissue proper.

The tissue pCO_2 electrode obviously measures the pCO_2 that exists on the surface of the brain. This tension is not necessarily identical with the average tissue pCO_2 of the grey matter. If a uniform average pCO_2 is assumed for the tissue proper in a given cortical area, the first problem to be dealt with is the assessment of the constancy of the pCO_2 as measured on the pia-arachnoid membrane in that area. The pia-arachnoidea is not representative of the actively metabolizing tissue and further contains arteries and veins, which must have a marked influence on the pCO_2 in their neighbourhood. Thus, if placed directly across a vein, an electrode with a diameter of the same size as that of the vein would measure a value similar to the venous pCO_2. It is then clear that the ideal electrode is one that measures a mean value of all different tensions on the surface.

If the requirements for constancy and reproducibility in measuring the surface pCO_2 are satisfied, the second problem is to correlate the pCO_2 as measured on the surface of the brain with that of the cortical grey matter proper. A useful method for assessing the latter tension implies application of diffusion laws for calculating the average tissue tension (THEWS 1953, 1960, KETY 1957). This method requires information on the arterial and venous pCO_2 as well as on the metabolic production of carbon dioxide. For practical reasons, measurements of venous pCO_2 and cortical metabolic rate can only be made if venous blood, coming from different cortical areas, is used. This fact is bound up with the third problem, that of regional differences in pCO_2 between different cortical areas. It is clear that if such differences exist between regions which contribute to the common venous pool, the correlation between the tissue pCO_2 and the surface pCO_2 will be hard to establish.

The present work yielded certain information on the problems outlined above. First, the reproducibility obtained on the same cortical spot shows that a reasonably constant pCO_2 exists for relatively long periods in a given cortical area provided that the animal is kept in a respiratory and circulatory steady state and that certain experimental conditions are fulfilled. These conditions are moderate electrode pressure, a tissue with a moist and undamaged surface and without signs of swelling and, finally, application of the electrode to an area which is void of large superficial veins. It is also indicated that the application of the electrode as such does not interfere with the measuring conditions. Second, the fact that the influence of the superficial vessels upon the value of pCO_2 obtained is confined to the largest veins reduces the importance of this influence since these veins can be easily avoided. An attempt to minimize the influence by an increase of the diameter of the electrode involves the drawback that the diameter becomes too great in relation to the size of the gyri. Third, the reproducibility of the pCO_2 values obtained in the investigated cortical areas indicates that, in the experimental conditions, the cerebral cortex can be looked upon as a rather uniform organ as far as the pCO_2 is concerned. It has previously been demonstrated that there are

regional differences in cortical blood flow in the cat (KETY 1960), which makes it probable that small differences in pCO_2 do in fact exist. Such differences, which may be so small that they escape detection with the present method, however, are of minor importance for calculations pertaining to the acid-base metabolism of the tissue. The reproducibility found seems also to be sufficient for a correlation between the pCO_2 recorded on the surface of the brain and that existing in the tissue proper, even if the determination of the latter involves measurements on cortical blood derived from a large cortical area.

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Aspects on the Glucose Metabolism of the Hypothalamus and the Pituitary in Goats

By

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Abstract

Andersson, B., S. Larsson and F. Pocchiari. *Aspects on the glucose metabolism of the hypothalamus and the pituitary in goats.* Acta physiol. scand. 1961. *51.* 314—324. — With the aid of ^{14}C-labelled glucose certain aspects of glucose metabolism have been studied in different parts of the hypothalamus and in the posterior and anterior pituitary. The metabolic pattern of the hypothalamus differs from that of the pituitary. Also within the hypothalamus certain differences exist with regard to the fate of the glucose in the incubation medium. Large amounts of GABA are found in the hypothalamus and the posterior pituitary. Glutamine was formed in all samples studied. In the anterior pituitary, glutamine contributes to about 25 % of all amino acids formed from the radioactive glucose. In the hypothalamus and the posterior pituitary about 10 μg per 25 mg of tissue per hour of the glucose present in the incubation medium was converted into amino acids.
The findings are discussed in relation to previous biochemical and physiological studies on this subject.

Different parts of the hypothalamus have different levels of metabolic activities with respect to ^{32}P-turnover (Forssberg and Larsson 1954, 1955). In a previous study of the fate of ^{14}C-glucose different metabolic activities were observed in various parts of the rabbit brain (Chain *et al.* 1960 a).

The present investigation was undertaken to study the fate of generally ^{14}C-labelled glucose in particular parts of the hypothalamus of goats. The glucose metabolism of the posterior and anterior parts of the pituitary was also studied.

Abbreviation: γ — aminobutyric acid..... GABA.

Materials and methods

Generally labelled ^{14}C-glucose was obtained from the Radiochemical Centre, Amersham, England. The radioactive material was diluted to give a specific activity of 20 μC per mg.

The composition of the medium was as follows: NaCl 0.098 M, KCl 0.027 M, $MgSO_4$ 0.0012 M, KH_2PO_4 0.0004 M, Na_2HPO_4 0.0175 M and radioactive glucose 0.0056 M — 3.6 mC/mM at pH 7.3 (CHAIN *et al.* 1960 b).

Goats of different ages and sexes fed *ad libitum* were killed by decapitation. The parts of the brain and the pituitary to be studied were dissected out as quickly as possible. The lapse of time when the animals were killed until the samples were placed in the Warburg vessels was bout 10 min. The tissue samples were weighed on a torsion balance and transferred to 0.5 ml of chilled incubation medium in small Warburg vessels of about 4 ml total volume. The centre well of the vessels contained small rolls of filter paper soaked with 0.07 ml 30 % NaOH for the absorption of respiratory carbon dioxide.

The following samples were taken (Fig. 1):

1) The periventricular hypothalamic tisssue adjacent to the third ventricle. The sample included the paraventricular nucleus (1).

2) The ventromedial hypothalamic tissue including the ventromedial hypothalamic nucleus (2).

3) Parts of the posterior pituitary (5) consisting mainly of neurohypophysial tissue but mixed to some degree with intermediate lobe tissue.

4) Parts of the anterior pituitary.

In some cases the mammillary bodies (3) and the supraoptic region were also taken for incubation.

In all the experiments the tissue was aerobically incubated for 60 min at 37° C. The gas phase was pure oxygen and the oxygen consumption was measured every 15 min. The $^{14}CO_2$ was determined as described by VILLEE and HASTINGS (1949).

After incubation, the medium was immediately transferred to paper for chromatography and the tissue was treated as described by CHAIN *et al.* (1960 a). The solvents for the chromatography were also the same as those used by these authors. All chromatograms were scanned quantitatively by the automatic scanning device (CHAIN *et al.* 1956, FRANK *et al.* 1959).

In some experiments the insoluble residue was treated with 0.5 N NaOH for 36 hours at 50° C, and transferred to platelets and counted. In the other experiments this fraction was hydrolysed for 48 hours with 5 N HCl at 100° C in a sealed tube, the acid being removed by several re-suspensions in water and dried over KOH. The dried residue was dissolved in water and chromatographed bidimensionally as described by BELOFF-CHAIN *et al.* (1959).

After scanning, the chromatograms from the hydrolysed insoluble residue were sprayed with ninhydrine in an acetone solution (0.1 % w/v).

Results

The oxygen uptake of the different parts of the hypothalamus and of the posterior and anterior pituitary is shown in Table I A and B. Very small differences existed in oxygen uptake between the various parts of the diencephalon. The oxygen consumption of the posterior pituitary was slightly lower, and the anterior pituitary had an oxygen consumption which was still lower than the hypothalamic areas. Table I also shows the $^{14}CO_2$ and ^{14}C-lactate formation.

Table I A. Oxygen consumption, $^{14}CO_2$- and ^{14}C-lactic acid formation in different parts of the hypothalamus and the pituitary in goat

Oxygen consumption expressed as μl per 25 mg of tissue (wet wt.) per hour.
$^{14}CO_2$ and ^{14}C-lactic acid expressed as μg glucose converted per 25 mg of tissue per hour.
Number of experiments given in parenthesis. Mean values ± S. E.

	Periventr. tissue	Ventromed. tissue	Supraopt. tissue	Mammillary bodies	Posterior pituitary	Anterior pituitary
O_2	38.7 ± 3.1 (14)	34.0 ± 3.3 (10)	36.3 ± 2.7 (4)	35.2 ± 2.9 (5)	30.3 ± 3.2 (10)	26.1 ± 2.6 (9)
$^{14}CO_2$.....	11.9 ± 1.0 (14)	10.8 ± 1.9 (10)	9.9 ± 1.1 (5)	17.6 ± 2.1 (5)	8.7 ± 1.6 (10)	2.3 ± 0.3 (8)
^{14}C-lact. acid	102.3 ± 7.2 (13)	89.8 ± 4.3 (10)	100.4 ± 6.8 (5)	125.0 ± 10.2 (5)	72.3 ± 5.1 (10)	30.3 ± 1.3 (8)

Table I B. Oxygen consumption and $^{14}CO_2$ production expressed as μM per 25 mg of tissue (wet wt.) per hour

Tissue	μM O_2	μM CO_2	$\frac{CO_2}{O_2}$
Periventr.	1.73	0.40	0.23
Ventromed.	1.52	0.36	0.24
Supraopt.	1.62	0.33	0.20
Mammillary b.	1.57	0.59	0.37
Poster. pit.	1.35	0.29	0.21
Anter. pit.	1.17	0.08	0.07

These values largely followed the course observed for oxygen, except for the anterior pituitary where not only absolutely but also comparatively both these components were low. When regarding the "^{14}C" RQ's (Table I B), that is the ratio between the CO_2 produced by the glucose in the medium and the total oxygen consumed there is very little difference between the various parts of the hypothalamus and the posterior pituitary. The anterior pituitary, however, had a "^{14}C" RQ which was very low — 0.07. The true RQ of the anterior pituitary was found to be about 0.85—0.90.

The quantities of the amino acids formed by the glucose in the incubation medium are shown in Table II and III. They amount to about 10 % of the glucose metabolized in the hypothalamus and in the hypophysis.

The total amount of amino acids formed was highest in the hypothalamus and in the posterior pituitary. The quantitative distribution of the amino acids formed in the hypothalamus showed certain differences (Table II). In each of

Table II. Amino acid formation from metabolised glucose in different parts of goat hypothalamus and in posterior and anterior pituitary

Results expressed as μg glucose converted per 25 mg of tissue (wet wt.) after 1 hour incubation at 37° C in O_2 in 0.5 ml medium (glucose concentration 0.1 %; total radioactivity 10 μC per vessel). Mean values ± S. E.

Tissue	Alanine	Aspartic acid	Glutamic acid	GABA	Glutamine	Arginine	Residue	No. of experiments
PV	1.4 ± 0.1	0.9 ± 0.1	6.0 ± 0.7	3.5 ± 0.3	1.2 ± 0.3	0.9 ± 0.2	0.6 ± 0.1	14
VM	1.3 ± 0.3	0.5 ± 0.1	4.4 ± 0.6	2.6 ± 0.5	1.1 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	10
SO	1.3 ± 0.2	0.7 ± 0.1	3.8 ± 0.6	2.7 ± 0.4	0.8 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	5
CM	1.2 ± 0.2	1.4 ± 0.2	6.1 ± 0.8	3.2 ± 0.4	1.1 ± 0.2	traces	0.2 ± 0.05	5
PP	0.7 ± 0.2	0.8 ± 0.1	4.1 ± 0.5	1.0 ± 0.03	0.7 ± 0.1	0.7 ± 0.2	1.1 ± 0.2	10
AP	0.9 ± 0.1	0.4 ± 0.05	0.7 ± 0.1		0.9 ± 0.2	0.4 ± 0.05	1.5 ± 0.2	8

PV = periventricular, VM = vetromedial, SO = supraoptic, CM = mammillary bodies. PO = posterior pituitary and AP = anterior pituitary tissue.

Table III. The percentage distribution of ^{14}C in amino acids formed by uniformly labelled glucose in different parts of the hypothalamus and the pituitary

The same abbreviations as in Table II are used.

Tissue	Alanine	Aspartic acid	Glutamic acid	GABA	Glutamine	Arginine
PV	10.0	6.5	43.2	25.2	8.6	6.5
VM	12.6	4.9	42.7	25.2	10.7	3.9
SO	12.6	6.8	36.9	26.2	7.8	9.7
CM	9.2	10.8	46.9	24.6	8.5	
PP	8.8	10.0	51.2	12.4	8.8	8.8
AP	27.3	12.1	21.2		27.3	12.1

these parts and in the posterior pituitary more ^{14}C-glutamic acid was formed than any of the other amino acids. In the anterior pituitary, the amounts of alanine, glutamic acid and glutamine were about equal. The formation of GABA was higher, both absolutely and proportionally in the periventricular and ventromedial tissues (and in the supraoptic tissue) than in the pituitary samples (Table II and III), where only occasionally traces were found in the anterior pituitary.

Glutamine formation from the glucose in the medium was observed in all the samples studied. In the anterior pituitary it takes up a large proportion of the total aminoacids found to be labelled with ^{14}C (Table III).

A radioactive spot on the bidimensional chromatogram, identified as arginine,

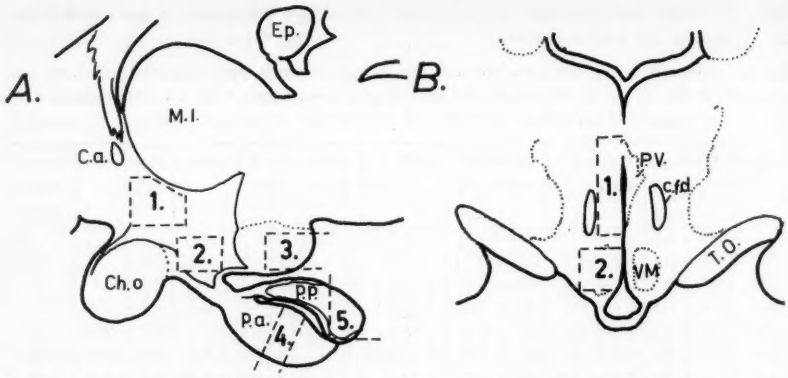

Fig. 1. *Diagrams of sagittal (A) and transverse (B) sections through the diencephalon of a goat, indicating the areas taken for incubation.*
1. Periventricular tissue, 2. Ventromedial tissue, 3. Mammillary bodies, 4. Anterior pituitary, 5. Posterior pituitary.
Ep. = Epiphysis, M.I. = Massa intermedia, C.a. = Anterior commisure, a.p. and p.p. = anterior and posterior pituitary, Ch.o. = Optic chiasma, P.V. = Nucleus paraventricularis, V.M. = Nucleus ventromedialis, C.f.d. = Columna fornicis descendens, T.O. = Tractus opticus.

was found in extracts from the various parts of the hypothalamus and the pituitary, the periventricular and supraoptic tissues forming the greatest amount (0.9 and 1.0 μg). The percentage distribution of this substance is shown in Table III. One other, still not identified spot on the chromatogram was found radioactive. It was mostly observed in extracts from the periventricular tissue. After incubation of particularly the anterior pituitary but also the ventromedial parts of the hypothalamus small amounts of ^{14}C-proline was found.

The total radioactivity of the insoluble residue was highest in the samples from the pituitary (Fig. 1). In the hypothalamic samples studied, the periventricular tissue had the highest incorporation of ^{14}C in this fraction. Due to the relatively low radioactivity in the insoluble residue no strict quantitative data of the protein bound amino acids were obtained. Qualitatively, however, it seemed as if glutamic acid and alanine were radioactive in this fraction both in samples from the hypothalamus and from the pituitary.

Using the technique mentioned previously radioactive glycogen and oligosaccharides were also found in the extracts from the hypothalamus and the pituitary. Also in this case the anterior pituitary contained the greatest amount, followed by the posterior pituitary.

Discussion

In a recent study CHAIN *et al.* (1960 a) found differences in the fate of radioactive glucose incubated with different parts of the rabbit brain, particularly in the distribution of certain amino acids. The hypothalamus divided into 3

samples were studied and also the cerebral and cerebellar cortici. Enough tissue for incubation was not obtained if a further dissection was performed. Instead, in the present study, brains from goats were used since their larger size permitted dissection of discrete parts of the hypothalamus. The goat has also been used in several physiological studies of the hypothalamus (ANDERSSON *et al.* 1958).

The values of the oxygen consumption of the hypothalamic samples agree well with those obtained by CHAIN *et al.* (1960 a) and largely with those found by SETCHELL (1959) on sheep brain pieces. The oxygen consumption was found to be highest in the periventricular and supraoptic tissue; histologically these two parts of the hypothalamus have much in common. The explanation for the slightly higher oxygen values in these two tissues as compared to the ventromedial tissue remains unknown. However, it has been found by histochemical studies that both the paraventricular and supraoptical nuclei contain more phosphatases than surrounding parts of the hypothalamus (ERÄNKÖ 1951).

Generally, the amounts of CO_2 and lactic acid formed by the glucose in the medium followed the oxygen consumption. This became more evident by regarding the "^{14}C" RQ's. The only exception to this was the anterior part of the pituitary where the "$^{14}CO_2$" RQ only was 0.07. ROBERTS and KELLER (1955) studied the oxygen consumption and lactic acid formation in hypothalamic and pituitary tissue before the administration of adrenaline or cortisone. They found a normal oxygen consumption which showed very small differences among the various parts studied. In these experiments, however, one has to consider the necessary inaccuracy involved in obtaining the tissue samples in the rat because of its small brain. In the present experiments small differences existed between the hypothalamic samples, but the anterior and also the posterior pituitary had a significantly lower oxygen consumption. As found by CHAIN *et al.* (1960 a) small quantities of potassium will increase the oxygen consumption in the hypothalamus as well as in the cerebral and cerebellar cortici. This stimulating effect on brain slices was first observed by ASHFORD and DIXON (1935) and DICKENS and GREVILLE (1935). The values for the CO_2-formation from the glucose of the medium agreed well with those obtained from the hypothalamus in an earlier study (CHAIN *et al.* 1960 a), where it was shown that while potassium increased the oxygen consumption in the hypothalamus, the formation of CO_2 from the medium-glucose diminished. In the cerebral cortex, however, the CO_2-formation was increased. It thus seemed that the addition of potassium partly increased the endogenous oxidation of the hypothalamus. In the present study, as mentioned previously, this effect seemed to be still more pronounced in the anterior pituitary where the "^{14}C" RQ was only 0.07. The true RQ of the hypothalamus was approximately 1, and even if the corresponding figure for the anterior pituitary was less (0.85—0.90), one can still state that the endogenous oxidation was very pronounced in this tissue. The same tendency was met in the study by BELOFF-CHAIN *et al.* (1959) where

the glucose metabolism in whole pituitary of rats had been observed. Here, however, the true values for the anterior pituitary were masked by the contamination of posterior pituitary tissue. Further, CHAIN *et al.* used a phosphate saline buffer without potassium which could explain why their values on the oxidation of endogenous products were less than those reported here, where potassium was used in the buffer. The mammillary bodies being an extrahypothalamic mesencephalic tissue had a "^{14}C" RQ which was highest of the tissues studied and resembled more the behaviour of the cerebral cortex in the study by CHAIN *et al.* (1960 a).

In the present experiments on the hypothalamus the labelling of the different amino acids from the glucose in the incubation medium followed the same gross pattern as that previously reported (CHAIN *et al.* 1960 a, TOWER 1959). Within the hypothalamus there were certain distribution differences (Table II and III). These were not big but they were consistent from experiment to experiment. In the earlier studies on the rabbit hypothalamus such variations were not observed (CHAIN *et al.* 1960 a). In the present study the observed differences in the distribution pattern among the amino acids probably depended on the further dissection of the hypothalamus. As was discussed by ANDERSSON *et al.* (1958) various parts of the hypothalamus of the goat give different responses to electrical stimulation. Thus, the ventromedial and the periventricular parts of the hypothalamus have different physiological functions which might explain the differences in amino acid distribution. Regarding the earlier results by CHAIN *et al.* (1960 a) and the present ones it is evident that the brain is far from a biochemical unit. Such a conjecture is more justified in view of the earlier results showing that hunger and satiety caused profound differences in the levels of ^{32}P-turnover and the formation of phosphorus containing high energy substances in small adjacent areas of the hypothalamus in the rat (FORSSBERG and LARSSON 1954, 1955).

In all the hypothalamic tissues studied glutamic acid was formed in larger amounts than any other amino acid. This was in agreement with the earlier studies by CHAIN *et al.* (1960 a) and by TOWER (1959). A discussion as to the central position of glutamic acid in the brain metabolism due to its ability to undergo oxidation, amidation, transamination and decarboxylation has been given by ANSELL and RICHTER (1954).

The present experiments have also confirmed earlier findings that the GABA-formation of the hypothalamus is high (CHAIN *et al.* 1960 a). Of the total amount of amino acids formed from the glucose in the medium, GABA took up about 25% in the hypothalamic samples. The mammillary bodies, in spite of its close anatomical relation to the hypothalamus, only had a GABA-formation which was about 15 % of the total amino acids formed. This amount was more related to that found in the cerebral cortex by CHAIN *et al.* (1960 a). It is of interest to note that the posterior pituitary was able to convert glucose into GABA, while only occasionally traces of this amino acid could be found in the anterior

pituitary. Since the discovery that the mammalian brain contains large amounts of GABA (ROBERTS *et al.* 1950, ROBERTS and FRANKEL 1950) this substance has received much attention. HAYASHI *et al.* (1956) reported that GABA and especially γ-amino-β-hydroxy-butyric acid inhibited convulsions induced in dogs by electrical or chemical stimulation. The literature concerning the mode of action of GABA and its derivatives is still controversal (PURPURA *et al.* 1959, CURTIS *et al.* 1959). The possible role of GABA or of related substances as a synaptic transmitter has not been proven by any direct experiments. However, there seems to be little ground for doubt that this type of substances are of some importance for the brain. Certain results have indicated that the GABA-content of the brain varies inversely with brain excitability (HÄKKINEN and KULONEN 1959). Preliminary experiments have also shown that certain tranquilizers selectively will influence the metabolic level of the hypothalamus, particularly the processes concerned with the GABA-formation (LARSSON 1960). The high rate of formation of GABA in the hypothalamus found earlier (CHAIN *et al.* 1960 a) and in the present study could partly be explained by the finding that the total amount of GABA is high in the diencephalon (ROBERTS and BAXTER 1959).

Glutamine is closely related to glutamic acid and GABA. While GABA is the decarboxylation product of glutamic acid, glutamine is the amid of glutamic acid. Considerable amounts of glutamine will be formed from the glucose in the incubation medium when potassium is present in the substrate (CHAIN *et al.* 1960 a). Without potassium only traces of glutamine were formed in the hypothalamus while the cerebral and cerebellar cortici were still able to produce certain amounts of the substance (CHAIN *et al.* 1960 a). Also in the present study, where exclusively a potassium containing medium was used, glutamine formation was high, not only in the hypothalamus but also in the pituitary. As glutamine is the amide of glutamic acid one would assume that a high glutamic acid formation also should lead to a high glutamine formation, provided ammonia was available. The importance of accessability of ammonia for glutamine formation has been pointed out by TOWER (1959). In the present experiments it also seemed as the glutamine formation followed glutamic acid one except in the case of the anterior pituitary. Here more glutamine is formed after incubation than glutamic acid, which could imply a larger excess to ammonia here. BELOFF-CHAIN *et al.* (1958) did not observe any glutamine formation in total pituitary from the rat, that is glucose in the medium converted into glutamine. In their experiments, however, a potassium free medium was used, which will prevent or diminish the glutamine formation in the different parts of the brain (CHAIN *et al.* 1960 a). In the anterior pituitary the alanine production from the glucose in the medium was also found to be comparatively high, especially when regarding the percentage distribution of the amino acids. The high rate of formation of alanine and glutamine in the anterior pituitary, in spite of its comparatively low "exogenous" activity totally, might be explained

by the fact that these substances both are constituents of the active principles of the anterior pituitary (Li *et al.* 1958).

In the active peptide principles of posterior pituitary hormones, synthesised by hypothalamic neurones, glutamine is also present (Du Vigneaud 1955, Bartlett *et al.* 1956). In the present study, however, no particularly high values for glutamine were found either in the hypothalamic or posterior pituitary samples. Slightly contradictory to the discussion above about glutamine as dependent upon the glutamic acid formation are the observations discussed by Tower (1959) that the two substances not necessarily have to parallel each other under certain abnormal conditions of the brain. In the hydrolysed insoluble residue ^{14}C-glutamine was found in all the samples studied.

As mentioned previously ^{14}C-arginine was found after incubation with ^{14}C-glucose on the chromatograms from extracts of the two parts of the pituitary and from the periventricular and supraoptic regions, whereas this substance was not observed consistently in extracts from the other parts of the hypothalamus. In this connection it is of interest to note that Rafelson *et al.* (1951) and Winzler *et al.* (1952) have found that *in vitro*, essential and non-essential amino-acids incorporated ^{14}C from glucose to about the same extent in the brain. In the present experiments the appearance of radioactive arginine was encountered in the same areas that have neurosecretory activities. Howe (1959) studied the distribution of arginine-positive material in the pituitary histochemically. In this study it seemed as if a histochemical reaction for arginine followed the location of neurosecretory material, and as mentioned above, in the present study, the formation of arginine from glucose appeared to be located in the same areas.

The anterior pituitary seemed to have its own metabolic pattern and the most pronounced differences from the other tissues studied were the relatively high formation of radioactive alanine and glutamine. Further, a comparatively high ^{14}C-activity in the "insoluble residue" was also found in this part. The amount of ^{14}C in the "insoluble" fraction was largely found to be in the protein-bound amino acids. Besides, the anterior pituitary, high activites in this fraction were found in the posterior pituitary and in the periventricular and supraoptic parts of the hypothalamus. This might depend upon the special demands of these parts in building up hormonally active principles and neurosecretory material. Also the other parts of the hypothalamus studied contained some ^{14}C-activity in the protein fraction. This was in agreement with the studies by Rafelson *et al.* (1951) and Winzler *et al.* (1952). They found that when brain slices were incubated with radioactive glucose or the substance was injected into animals there was a rapid formation of ^{14}C-amino acids in the brain, both free and protein bound.

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The Effects of Moderate Sleep Deprivation on the Habituation of Autonomic Response Elements

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Abstract

SCHOLANDER, T. *The effects of moderate sleep deprivation on the habituation of autonomic response elements.* Acta physiol. scand. 1961. *51.* 325—342. — The phasic responses and the prestimulus values of the electrodermal activity, the pupillary size and the pulse rate, as well as the prestimulus respiration rate were recorded during monotonously repeated auditory stimulation. Intra-individual comparisons were made between the results obtained after one night of wakefulness and after normal sleep. Among the recorded variables only two, viz. the response amplitudes (*i. e.* maximal post-stimulus changes) of the electrodermal activity and the pupil were influenced in a statistically significant way by the sleep deprivation. The habituation of the response amplitude of the electrodermal activity was delayed and the mean level of the pupillary response amplitude was increased. It was assumed that these effects were due to a conflict between a wish to stay awake and the successively increasing sleepiness induced by the sleep deprivation and the monotony of the experimental situation.

Remarkably few physiological changes have been observed during moderate sleep deprivation. Reviews of previous research on this subject have been published by KLEITMAN (1939), TYLER, GOODMAN and ROTHMAN (1947) and BJERNER (1949).

FREEMAN (1932) in studying performance found that the efficiency in different tasks could remain at a normal level for days following serious loss of sleep but that the muscular tension accompanying the work was higher under such circumstances. In an investigation which is specially relevant in the present context, BURCH and GREINER (1958) studied the electrodermal response (EDR)[1] during a prolonged wakefulness of 30 hours.[2] They included two different aspects of the EDR in their study. The "non-specific" response was measured "as the number of responses per unit time which occur due to internal stimuli, or to subliminal external stimuli not apparent to the observer". This nonspecific EDR showed a progressive depression with prolonged wakefulness. On the other hand the "specific" response (measured in per cent of the amplitude during a control period) to a mild electric shock either became depressed or increased successively during the experiment. The authors assumed that the depression of the specific response was due to "a condition approximating sleep" whereas the increase of the response reflected the high amplitudes which they had observed "during deep drowsiness and very light sleep". The distinction between "a condition approximating sleep" and "very light sleep" is unclear. However, it is also stated, that the increase of the response amplitudes was obtained "in an active social situation with continued arousal and pressure for performance". This is important information which indicates that this response pattern might be a similar phenomenon to the above-mentioned increase in the muscular tension obtained by FREEMAN (1932), viz. a reflection of the effort to maintain normal efficiency of performance in spite of the sleep loss.

The most consistent finding during sleep deprivation has been a decrease of the alpha activity of the EEG (KLEITMAN 1939, TYLER *et al.* 1947, BJERNER 1949, ARMINGTON and MITNICK 1959). Two different interpretations of this result have been proposed. TYLER *et al.* (1947) found that the decrease of the alpha activity was followed by an increase of the faster activity. They suggested that these changes might be caused by the effort in staying awake. On the other hand, BJERNER (1949) recorded EEG and pulse rate parallel to a serial reaction test. He showed that "delayed actions" in the reaction test were significantly associated with decrease of the number of alpha waves and of the pulse rate. Similar results were obtained by ARMINGTON and MIT-

[1] The term used by the authors is "galvanic skin response" (GSR) which is rather misleading (*cf.* WOODWORTH and SCHLOSBERG, 1955, p. 137). Because of this, and to make the description uniform, the more appropriate term "electrodermal response" (EDR) will be used in the following to describe acute changes in the skin resistance. The phenomena underlying the measurements of skin resistance and skin potentials will be called "electrodermal activity" (EDA).

[2] Unfortunately the paper is only a condensed version of an article published "under the auspices of N. A. T. O.". The complete version has not been available. Consequently we lack certain important information about the experimental conditions, the number of stimuli used, the intervals between stimuli etc.

NICK (1959) during an auditory vigilance task. According to these authors the over-all decrease of the alpha activity is caused by sleepiness. They also suggest that possible impairment of performance may be due to short periods of sleep or a condition like sleep. This "lapse" hypothesis is very attractive. Yet it seems somewhat inadequate to discard the interpretation presented by TYLER *et al.* (*cf.* WILLIAMS, LUBIN and GOODNOW 1959, p. 22). These authors emphasize that their subjects were highly motivated to stay awake. In addition the portions of the EEG records showing sleep activity were not used in the analysis. In this respect the experimental approach was different from that of BJERNER (1949) and of ARMINGTON and MITNICK (1959). Accordingly it seems probable that both the sleepiness and the effort to stay awake may decrease the alpha activity. The causal connection would be dependent on the experimental conditions.

Rationale and aim of the present investigation

In a previous investigation (SCHOLANDER 1960 b) it was shown that the habituation of EDRs and pulse rate responses was delayed when the subject was preoccupied with a simple sighting device. Ordinarily this device makes it possible for the subject to control his position in relation to a camera for recordings of the pupillary reactions. It was assumed that the delay of habituation obtained was due to increased alertness induced by the preoccupation with the sighting device.

In the present study the use of the pupillary camera could be expected to induce what might be called an "experimental conflict situation". It is a common observation that monotonous and prolonged repetition of a stimulus tends to induce a successively increasing drowsiness. It was assumed that this drowsiness would be more pronounced and appear earlier after a night without sleep than under ordinary circumstances. On the other hand, the above-mentioned results have indicated that the use of the pupillary camera counteracts the successive decrease of alertness consequent upon stimulus repetition. It is also obvious that the subjects' "anxiousness" to preserve good pupillary recordings would be a further incitement to stay awake.

It has been the aim of the present investigation to study the effects of this "conflict" between sleepiness and the wish to stay awake on the habituation of different autonomic response elements. The prestimulus values and the phasic responses of the electrodermal activity (EDA), the pupil and the pulse rate, as well as the prestimulus respiration rate were recorded during monotonously repeated auditory stimulation. Comparisons were made between the results obtained after one night of wakefulness and after normal sleep. In addition the pupillary recordings gave an opportunity to follow the tendency of the subjects to shut their eyes throughout the experiments. It was natural to assume that this tendency would be correlated to the degree of sleepiness.

It was predicted that after sleep deprivation the "conflict" between sleepiness and wish to stay awake would delay the habituation and increase the level of the phasic reactivity when compared with the results obtained after normal sleep. Previous investigations have given few clues as to the possible effects on the prestimulus values. Consequently in this case no directional hypotheses were stated.

Methods

1. Laboratory and apparatus: The laboratory and apparatus used in the present study have been described in detail elsewhere (DUREMAN 1959, SCHOLANDER 1960 a). The electrodermal activity (EDA) was recorded according to NICHOLS and DAROGE (1955). In tabulating the EDA-data the conductance (micromhos) was used. A special camera which employs intermittent infrared light (DUREMAN, SCHOLANDER and SÄLDE 1959) recorded the pupillary reactions. The camera was coupled to the timer which initiated the sequence of the stimuli. In tabulating the pupillary data, square measure was used. This gave a better idea of phasic pupillary increments starting from large prestimulus pupils than linear measure (*cf.* DUREMAN and SCHOLANDER 1959). In addition the pictures taken when the subject kept his eyes shut could be counted.[1] The film speed was 5 pictures per second and the number of pictures taken during each trial varied from 18 to 20. However, uniformity was achieved by limiting the above-mentioned counting to the first 15 pictures of each trial.

The pulse and respiration rates were recorded by means of pneumographic devices (*cf.* DUREMAN 1959, p. 100). The prestimulus respiration rate was obtained by counting the number of cycles occurring within a 20 sec. interval preceding each stimulus. These values were multiplied by three to yield respiration rate per minute. The somewhat cumbersome counting of the pulse waves was abandoned in favour of a method for obtaining a direct, continuous recording (*cf.* SCHOLANDER 1960 a).

The stimulus, a "white noise" signal with a duration of 0.1 sec and an intensity of 105 db above threshold, was administered binaurally through headphones.

2. Subjects: The subjects were 12 students, 6 males and 6 females, aged 21 to 27 years. Each subject was present for 35 min on each of two days separated by a three-day interval. To control as far as possible the influence of diurnal variations each subject returned at the same time of the day.

3. Procedure: The subjects were divided at random into two groups of equal size. In one group the first experiment was undertaken after one sleepless night (in the following called "no sleep" condition) and the second experiment after a night with normal sleep (in the following called "control" condition). In the other group the order of the sessions was reversed, *i. e.* first the control experiment and then the "no sleep" experiment. The subjects were given standard instructions. They were informed that the investigation was centred around the study of the influence of prolonged wakefulness on autonomic responses to repeated auditory stimulation. They were told that during the day and night before the control experiment they should follow their normal routine. They should sleep without any hypnotics and avoid stimulants, *e. g.* coffee and tea on the morning of the experiment. The subjects were told to keep awake during

[1] In the following such pictures will be called "closed-eye" pictures. They were not only due to ordinary eye blinks. Especially after sleep deprivation the subjects tended to shut their eyes during longer periods and sometimes there was clear evidence that these periods were due to sleep (*cf.* p. 6).

the day and night before the other session but apart from this the instructions were exactly the same as for the control experiment.[1]

It was further emphasized that continuous active cooperation on the part of the subject was necessary to guarantee optimal quality of the pupillary recordings. The subjects were instructed to maintain exactly the same position throughout the whole session by concentrating on the small red twinkling bulb which served as fixation point and which was seen through a simple sighting device on the camera. This part of the instructions was considered especially important in view of the results obtained in previous investigations (SCHOLANDER 1960 a, b).

Each session began with a period of 5 min during which the subject could relax and become accustomed to the apparatus. The flash tubes and the stroboscope of the pupillary camera make a faint clicking sound. To ensure habituation to this sound the apparatus was switched on and off 15 times with 5 to 10 sec intervals before 5 control exposures were made with the camera.

Immediately after this period the subject was given 30 "white noise" stimuli with intervals varying at random between 20 and 40 sec according to a sequence uniformly applied to all subjects during both sessions.

4. Treatment of data: All comparisons made in the present investigation were intra-individual. To simplify the treatment of data and to increase the reliability of the measurements, average values of successive blocks of 5 trials were computed. A distinction was made between 1) the so-called tonic activation or tonicity level, operationally defined as the prestimulus level and 2) the phasic reactivity. The latter was studied in terms of both the response amplitude, *i. e.* the maximal post-stimulus change, and the frequency of positive reactions during successive groups of five trials. For the comparison between the results obtained during the two sessions daily average values were used. As a gross measure of the change of the parameters within each session, individual "change indices", *i. e.* the average percentage divergences from the values at the beginning of the session were computed. First, the arithmetical mean prestimulus value or the mean response amplitude during the last 25 trials was expressed as a percentage of the average value during the first 5 trials. Then, to get the percentage change, the obtained value was subtracted from 100 (*cf.* SCHOLANDER 1960 a).

Non-parametric statistical methods were used in the analysis of the data. To test possible changes occurring within sessions, Friedman two-way analyses of variance by ranks (SIEGEL 1956, p. 166 ff) were applied. The differences between the two sessions were tested by means of Wilcoxon matched-pairs signed-ranks tests (ibid., p. 75 ff). As a rule the null-hypothesis (H_0) was rejected in favour of the alternative hypothesis (H_1) if the probability yielded by the above-mentioned tests was equal to or less than $\alpha = 0.05$. A one-tailed test of significance was applied whenever H_1 indicated the predicted direction of the difference. When the results were against the predictions or no directional hypothesis had been stated a two-tailed test was used.

Results

The individual recordings

Before giving an account of the group trends obtained in the present investigation, certain interesting observations made during the individual recordings should be mentioned. In accordance with the results obtained in a

[1] The observance of these instructions was not controlled but the subjects were relied upon to do their best. In any case possible divergences from the instructions would work against the given hypotheses and this possible source of error was therefore considered to be of minor importance.

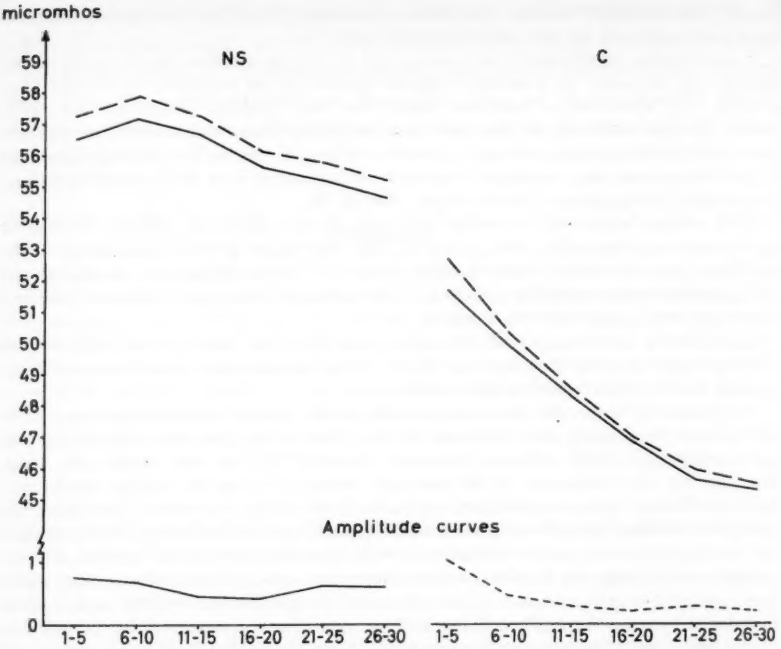

Fig. 1. The mean values of the prestimulus EDA (upper curves) and the EDR amplitude (lower curves) during successive groups of five trials during the "no sleep" (NS) and the control (C) condition.

previous investigation (SCHOLANDER 1960 a) the individual records of all variables showed considerable fluctuations. However, this irregularity was more pronounced during the "no sleep" than during the control condition. The phasic response amplitudes of the EDA and the pupil in particular varied after sleep deprivation. High peaks with amplitudes two or three times as great as those obtained during the first trial often alternated with absence of response. The fluctuations of the phasic response amplitudes showed a tendency towards increase during the latter stage of the "no sleep" experiment, but this tendency was not uniform. Moreover, the above-mentioned high amplitude peaks appeared completely irregularly in the different functions. Thus a high EDR amplitude was seldom paralleled by a high pupillary response amplitude.

In general the number of "closed-eye" pictures increased considerably during the last 15 trials of the "no sleep" experiment (*cf.* p. 14). This increase was due mainly to the tendency of many subjects to keep their eyes closed during successively longer periods. In fact, 3 subjects under the "no sleep" condition showed all external signs of sleep such as closed eyes, deep

Table Ia. Distribution of intra-individual differences in mean prestimulus EDA (in micromhos) between the "no sleep" (NS) and the control (C) experiment

NS—C	Increase in conductance during C.				Decrease in conductance during C.				Mean diff.	T	p
	> —40	—30	—20	—10	0	10	20	30 40			
n = 12	1				2	3	2	1 3	7.76	17	> 0.05

Table Ib. The sums of individual ranks computed from the mean prestimulus EDA during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial:	1—5	6—10	11—15	16—20	21—25	26—30	χ_r^2	Significance
NS	48.0	52.5	51.5	37.5	30.5	32.0	11.65	$p < 0.05$
C	64.0	51.5	40.5	41.0	29.0	26.0	23.87	$p < 0.001$

regular breathing and low muscular tension. During these sleep-like periods a successive decrease of the tonicity variables was seen. In addition the phasic reactivity usually decreased or disappeared but this finding was not constant.

Prestimulus EDA

In Fig. 1 is shown a graph of the average EDA-data during the two experiments. In the upper curves the continuous lines represent the prestimulus levels and the broken lines the maximal post-stimulus levels. Consequently the response amplitude is the vertical distance between these lines. The amplitude data are further illustrated in the lower curves. Successive groups of 5 trials are plotted along the base line, the corresponding mean values of the skin conductance (in micromhos) and the conductance change along the vertical axis.

A comparison of the curves shows that the average prestimulus EDA stays at a higher level and declines less during the "no sleep" than during the control condition.

To obtain a statistical estimate of these trends the individual average values of the prestimulus EDA during each of the two experimental sessions were first computed. Table Ia shows the distribution of the intra-individual differences between these average values. However, when a Wilcoxon matched-pairs signed-ranks test was applied a significant level was not reached ($T = 17$, $p > 0.05$, two-tailed).

To test the habituation tendency of the prestimulus EDA during each of the experiments the mean values to successive groups of 5 stimuli were computed and ranked intra-individually. The sums of ranks (Table Ib) decrease con-

Table IIa. Distribution of intra-individual differences in mean EDR amplitude (in micromhos) between the "no sleep" (NS) and the control (C) experiment

NS—C	Increase in mm² during C.				Decrease in mm² during C.					Mean diff.	T	p
	—2.0	—1.5	—1.0	—0.5	0	0.5	1.0	1.5	2.0	2.5<			
n = 12	1				1	2	2	4	2	1	0.69	13	< 0.025

Table IIb. The sums of individual ranks computed from the mean EDR amplitude during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial:	1—5	6—10	11—15	16—20	21—25	26—30	χ^2_r	Significance
NS	53.0	45.0	26.5	35.0	50.0	42.5	11.52	$p < 0.05$
C	65.5	35.0	41.5	40.0	39.5	30.5	17.72	$p < 0.01$

tinually during both sessions. When Friedman two-way analyses of variance by ranks were applied, the control condition yielded the highest value of χ^2_r ($\chi^2_r = 23.87$, $p < 0.001$ versus $\chi^2_r = 11.65$, $p < 0.05$).

Finally "change indices" (*cf.* p. 5) were computed to make gross comparisons between the habituation trends possible. The intra-individual differences between the conditions in these indices yielded an insignificant value of T ($T = 19$, $p > 0.05$, two-tailed).

Thus neither the mean level nor the habituation of the prestimulus EDA showed significant differences between the experimental sessions.

EDR amplitude

A comparison of the curves derived from the average EDR amplitudes (lower part of Fig. 1) reveals that the control curve starts from a somewhat higher initial value than the corresponding "no sleep" curve. However, the former shows a clear-cut downward trend during the first 20 trials whereas the latter keeps at almost the same level throughout the experiment.

The amplitude data were treated in the same way as the prestimulus EDA. As predicted, a Wilcoxon test applied to the intra-individual differences between the two conditions in the average EDR amplitude (Table IIa) yielded a significant value of T ($T = 13$, $p < 0.05$, one-tailed).

The sums of ranks derived from the mean EDR amplitudes during successive groups of 5 trials are shown in Table IIb. These rank sums decrease during the first part of the "no sleep" experiment but show an upward trend during the last 15 trials. This tendency was so marked that a significant χ^2_r value was

Table III. The sums of individual ranks computed from the frequency of positive EDRs during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial:	1–5	6–10	11–15	16–20	21–25	26–30	χ_r^2	Significance
NS	60.0	45.0	24.0	32.5	43.5	47.0	18.44	$p < 0.01$
C	61.5	41.0	39.5	38.0	34.5	37.5	11.43	$p < 0.05$

Table IVa. Distribution of intra-individual differences in mean pupillary response amplitude (in mm²) between the "no sleep" (NS) and the control (C) experiment

NS—C	Increase in conductance during C. < —0.8	—0.6	—0.4	—0.2	0	0.2	0.4	0.6	0.8 < Decrease in conductance during C.	Mean diff.	T	p
n = 11	1			1	1	3	2	2	1	0.49	13	< 0.05

Table IVb. The sums of individual ranks computed from the mean pupillary response amplitudes during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial:	1–5	6–10	11–15	16–20	21–25	26–30	χ_r^2	Significance
NS	46.0	41.0	34.0	38.5	46.5	46.0	3.09	—
C	50.5	34.5	37.5	45.0	48.5	36.0	5.62	—

obtained when the Friedman-test was applied ($\chi^2_r = 11.52$, $p < 0.05$). During the control experiment the corresponding rank sums show a more successive decline which is highly significant ($\chi^2_r = 17.72$, $p < 0.01$). When the difference in the habituation trend between the two sessions was tested by means of "change indices" a significant T-value was obtained ($T = 10$, $p < 0.025$, one-tailed).

It may be concluded that the "no sleep" condition induced a higher mean level and a less pronounced habituation of the EDR amplitude than the control condition.

Frequency of positive EDRs

The frequency of positive EDRs during successive groups of 5 trials was used as a complementary variable of the phasic reactivity. To make possible comparisons between the mean levels of this parameter the total number of

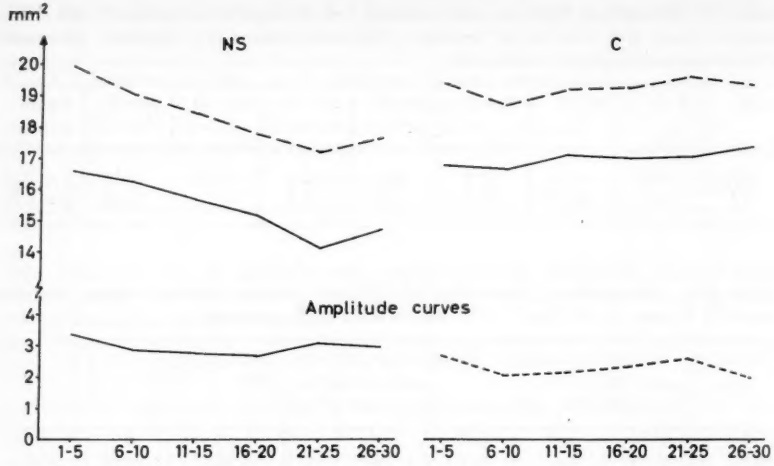

Fig. 2. The mean values of the prestimulus pupillary area (upper curves) and the pupillary response amplitude (lower curves) during successive groups of five trials during the "no sleep" (NS) and the control (C) condition.

positive responses during each session was computed. The intra-individual differences of these values between sessions were, however, insignificant ($T = 22$, $p > 0.05$, one-tailed).

The sums of ranks derived from the frequency of positive EDRs during successive groups of 5 trials (Table III) show trends similar to those obtained for the EDR amplitudes. However, the initial decrease and the subsequent increase of the rank sums during the "no sleep" experiment is here very pronounced and statistically highly significant ($\chi^2_r = 18.44$, $p < 0.01$). During the control condition a fairly even habituation trend was obtained ($\chi^2_r = 11.43$, $p < 0.05$). The intra-individual differences in the "change indices" between sessions were not significant ($T = 24.5$, $p > 0.05$, one-tailed).

The frequency parameter did not show any significant differences between the two sessions. However, the initial decrease and subsequent increase of this variable as well as the EDR amplitude during the "no sleep" condition is rather noteworthy. Evidently the wakefulness induced a marked tendency towards habituation during the first part of the experiment but this period was followed by a successively increasing phasic reactivity.

Prestimulus pupillary area

In Fig. 2, the pupillary data have been shown in the same way as the EDA-data in Fig. 1. All measurements are in mm². The average prestimulus pupillary area (upper curves) is somewhat smaller during the "no sleep" than during the control condition.

Further, the "no sleep" curve shows a slight downward trend whereas the level of the control curve is relatively constant.[1]

The intra-individual differences of the average prestimulus pupillary area between the two sessions did not reach a significant level ($T = 20$, $p > 0.05$, two-tailed). The sums of ranks derived from the mean values of the prestimulus pupillary area during successive groups of five trials revealed a decreasing trend during the "no sleep" condition and a slight increase during the control experiment. Neither of these trends were statistically significant ($\chi^2_r = 10.01$, $p > 0.05$ and $\chi^2_r = 4.91$, $p > 0.05$ respectively). According to the clearly significant value of T ($T = 12$, $p < 0.05$, two-tailed) obtained when a Wilcoxon test was applied to the corresponding intra-individual differences in the "change indices" the habituation of the prestimulus pupillary area appeared to be accentuated by the wakefulness. However, it should be emphasized that a number of interpolations of the pupillary data had to be made during the "no sleep" condition due to the "closed-eye" pictures. No safe conclusions can therefore be derived from the analysis of the present variable.

Pupillary response amplitude

The curves of the average pupillary response amplitudes (lower curves in Fig. 2) show differences which are in accordance with the predictions, *i. e.* a higher level and a less pronounced decline of the "no sleep" curve.

Among the intra-individual differences between the conditions in the pupillary response amplitude shown in Table IVa only two were against the predictions. A Wilcoxon test yielded a highly significant value of T ($T = 13$, $p < 0.025$, one-tailed). Thus in spite of the fact that the number of zero values which had to be recorded due to the "closed-eye" pictures was greater during the "no sleep" than during the control condition (*cf.* p. 14) the average pupillary response amplitude was still significantly higher during the "no sleep" experiment.

The habituation tendency was slightly more pronounced during the control experiment (Table IVb) but the intra-individual differences in the "change indices" did not reach a significant level ($T = 36$, $p > 0.05$, one-tailed).

Frequency of positive pupillary reactions

The statistical analysis of the frequency of positive pupillary reactions did not yield any significant intraindividual differences between the two sessions. A Wilcoxon test applied to the intra-individual differences in the total number of positive pupillary reactions yielded $T = 21.5$ ($p > 0.05$, one-tailed). Practically no habituation of the variable was obtained during the two conditions (during the "no sleep" experiment:

[1] It should be emphasized that during the "no sleep" condition the successively increasing number of "closed-eye" pictures made the pupillary recordings difficult. It was sometimes necessary to make interpolations between the prestimulus values obtained during the trials immediately before and after continuous series of "closed-eye" pictures, especially during the last ten trials. Whenever such periods made it impossible to obtain a value for the response amplitude a zero was recorded. It is, of course, questionable as to whether this was quite adequate. However, with the pupillary response amplitudes the possible bias introduced by the procedure was against the predictions. Nevertheless this variable yielded results in agreement with the predictions.

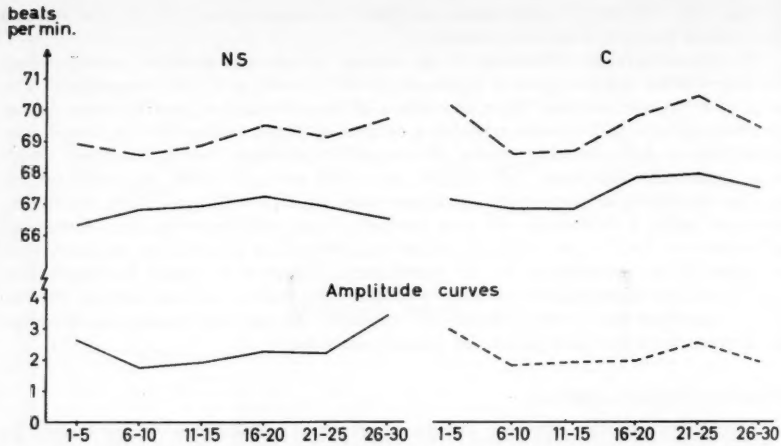

Fig. 3. The mean values of the prestimulus pulse rate (upper curves) and pulse rate response amplitude (lower curves) during successive groups of five trials during the "no sleep" (NS) and the control (C) condition.

$\chi^2_r = 1.92$ and during the control experiment: $\chi^2_r = 0.30$). Consequently the intra-individual differences in the "change indices" were also clearly insignificant ($T = 36$, $p > 0.05$, one-tailed).

Prestimulus pulse rate

A comparison between the curves derived from the average prestimulus pulse rate (Fig. 3, upper curves) reveals that the control curve keeps at a slightly higher level than the "no sleep" curve. There is little or no tendency towards habituation. In fact the control curve shows even a slight upward trend.

A Wilcoxon test applied to the intra-individual differences in the average prestimulus pulse rate yielded $T = 32$ ($p > 0.05$, two-tailed). The habituation tendency tested by means of Friedman analyses also yielded clearly insignificant values of χ^2_r ($\chi^2_r = 7.53$ during the "no sleep" and $\chi^2_r = 5.06$ during the control condition). Finally, the intra-individual differences in the "change indices" between the experiments were insignificant when a Wilcoxon test was applied ($T = 36$, $p > 0.05$, two-tailed).

Pulse rate response amplitude

The curves of the average response amplitudes of the pulse rate in Fig. 3 have certain common characteristics, viz. nearly equal levels during the first 25 trials. However, during the last 5 trials the "no sleep" curve rises and the other falls. Thus only the control condition yields a certain tendency towards habituation of this variable.

As seen in the graph the intra-individual differences in the mean response amplitude of the pulse rate did not reach a significant level ($T = 38$, $p > 0.05$, one-tailed). Furthermore, neither the "no sleep" nor the control condition yielded a significant degree of habituation ($\chi^2_r = 7.53$, $p > 0.05$ and $\chi^2_r = 6.29$, $p > 0.05$ respectively). When a Wilcoxon test was applied to the intra-individual differences in the "change indices" an insignificant T value was obtained ($T = 23$, $p > 0.05$, one-tailed).

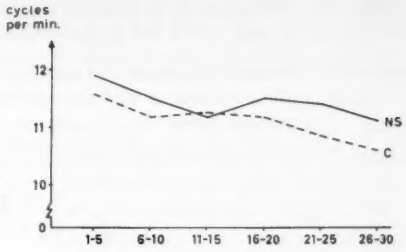

Fig. 4. The mean values of the prestimulus respiration rate during successive groups of five trials during the "no sleep" (NS) and the control (C) condition.

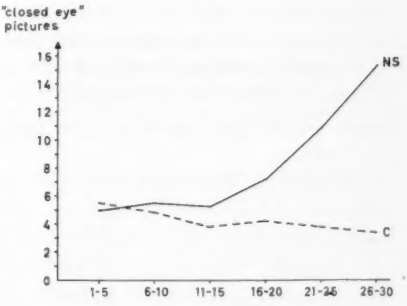

Fig. 5. The mean number of "closed-eye" pictures during successive groups of five trials during the "no sleep" (NS) and the control (C) condition.

Frequency of positive pulse rate responses

The statistical analysis of the frequency of positive pulse rate responses yielded results in good agreement with those obtained for the amplitude data, viz. no significant intra-individual differences in the total number of positive reactions ($T = 20$, $p > 0.05$, one-tailed) or in the "change indices ($T = 26$, $p > 0.05$, one-tailed). The habituation tendency was also clearly insignificant during both conditions ($\chi^2_r = 7.84$ during the "no sleep" experiment and $\chi^2_r = 5.99$ during the control experiment).

Prestimulus respiration rate

It appears from Fig. 4 that the level of the average prestimulus respiration rate is somewhat higher during the "no sleep" than during the control condition. Both curves fall slightly and are almost parallel throughout the experiment indicating a tendency towards habituation.

When Wilcoxon tests were applied to the intra-individual differences between the two conditions in the average prestimulus respiration rate and the "change indices", insignificant values of T were obtained ($T = 22.5$, $p > 0.05$, and $T = 22$, $p > 0.05$ respectively). According to Friedman analyses the habituation of the variable was insignificant both during the "no sleep" ($\chi^2_r = 5.26$, $p > 0.05$) and during the control experiment ($\chi^2_r = 4.79$, $p > 0.05$).

Number of "closed-eye" pictures

In Fig. 5, the mean numbers of "closed-eye" pictures during successive groups of 5 trials have been plotted separately for each of the two experimental

Table Va. Distribution of intra-individual differences in mean number of "closed-eye" pictures between the "no sleep" (NS) and the control (C) experiment

NS—C	Increase in number of "closed-eye" pictures during C.				Decrease in number of "closed-eye" pictures during C.						Mean diff.	T	p
	<—50	—40	—30	—20	—10	0	10	20	30	40 50<			
n = 12	1	1			1	3	2	1		3	28.1	16	< 0.05

Table V b. The sums of individual ranks computed from the numbers of "closed-eye" pictures during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial:	1—5	6—10	11—15	16—20	21—25	26—30	χ^2_r	Signif-icance
NS	31.0	38.0	33.5	44.5	44.5	60.5	13.32	$p < 0.02$
C	53.5	42.5	38.0	43.0	41.5	33.5	5.18	—

sessions. The curves keep at approximately the same level during the first part of the experiments. However, from the 15th trial onward the "no sleep" curve rises rapidly whereas the control curve falls.

The comparison between the total number of "closed-eye" pictures during the "no-sleep" and the control conditions was based upon intra-individual differences. The distribution of these differences is shown in Table V a. It appears that only two subjects had a greater number of "closed-eye" pictures during the "no-sleep" than during the control experiment. When a Wilcoxon test was applied a highly significant value of T was obtained ($T = 7$, $p < 0.025$, one-tailed).

It was assumed (*cf.* p. 3) that the number of "closed-eye" pictures would be correlated with the degree of sleepiness. Thus it was natural to assume that the number of such pictures would increase during the "no sleep" experiment. To test this last assumption the number of "closed-eye" pictures during successive groups of 5 trials was recorded. The obtained values were ranked intra-individually. In Table Vb the sums of these ranks are shown. In agreement with the corresponding curve in Fig. 5 they increase during the "no-sleep" experiment. This increase was highly significant when tested by means of a Friedman analysis ($\chi^2_r = 13.32$, $p < 0.02$). The corresponding decrease obtained during the control experiment did not reach a significant level ($\chi^2_r = 5.18$, $p > 0.05$).

Discussion

Among the variables recorded in the present investigation only the response amplitudes of EDA and pupil were influenced significantly by sleep deprivation.[1] Both these variables showed a higher mean level during the "no sleep" than during the control condition. However, the most important change in EDR amplitude was the delay of habituation obtained after sleep loss. This conclusion can be drawn because marked intra-individual differences were obtained between the mean values of all trials whereas the corresponding differences between the mean values of the first 5 trials were clearly insignificant (*cf.* the corresponding curves in Fig. 1). It seems that the habituation of the pupillary response amplitude was not affected by the sleep deprivation. On the other hand it should be emphasized that the successively increasing number of zero values due to "closed-eye" pictures made a safe evaluation of the habituation of this variable during the "no sleep" experiment practically impossible.

It is interesting to note that the sleep deprivation induced a pronounced decline of both EDR parameters during the first part of the experiment whereas an increase was seen during the last 15 trials. This rising trend coincided with a marked increase in the number of "closed-eye" pictures. As previously mentioned such pictures were recorded both during ordinary eye blinks and when the subjects kept the eyes shut during longer periods. Evidently these periods were sometimes due to sleep-like conditions. Three subjects showed all external signs of sleep during the last part of the "no sleep" experiment. Afterwards they admitted spontaneously that they had been sleeping. However, most of the subjects only reported pronounced sleepiness and increasing difficulty in keeping awake during the "no sleep" experiment. Under such circumstances it was impossible to decide whether the increased number of "closed-eye" pictures was caused by periods of sleep or simply difficulty in keeping the eyes open for other reasons. In fact there is some evidence that discomfort was the most common explanation. Irritation of the eyes, for instance, was a usual complaint after sleep deprivation. Three subjects appeared to fall asleep during the "no sleep" experiment, and all showed some kind av response decrement during the sleep-like periods. This is a similar response pattern to the one observed by BURCH and GREINER (1958) during a condition "approximating sleep". Thus it is reasonable to assume that an increase of the number of "closed-eye" pictures due to periods of sleep would have been followed by a decrease of the response amplitudes. On the contrary in the present investigation most subjects showed an increase in the

[1] All the intra-individual differences between the two sessions derived from the tonicity variables were insignificant. A possible positive relationship between the response amplitudes and the corresponding prestimulus values (*cf.* SCHOLANDER 1960 a, 1961 b) is therefore of minor importance in the present context.

EDR amplitudes during the last 15 trials of the "no sleep" experiment. This increase was mainly due to high amplitude peaks resembling those observed by BURCH and GREINER (1958) during "deep drowsiness and very light sleep". Moreover the mean frequency of positive EDRs during successive groups of five trials also increased.

According to the premisses, both the delayed habituation of the EDR amplitude and the increased mean level of the pupillary response amplitude after sleep deprivation might be explained by the conflict between a successively increasing sleepiness and a wish to stay awake. BURCH and GREINER (1958) obtained a progressive increase in the EDR amplitude during similar experimental conditions, *i. e.* lack of sleep combined with "continued pressure for performance". The authors related this response pattern to what they called a "cortical release". This concept is vague and misleading. LÖWENSTEIN and LOEWENFELD (1952) found that habituation of the pupillary light reflex during monotonously repeated light stimuli progressed in a peculiar rhythmical way. After an even decrement during the first 6 stimuli the response amplitudes began to alternate in a wave-like manner. The amplitudes of these waves progressively increased. They were more pronounced if the subject was tired at the beginning of the experiment. It was suggested that the monotonous repetition of the light stimulus induced sensations of fatigue which in a feed-back loop were counteracted by centrally evoked sympathetic impulses.

It has been pointed out that the brain stem reticular formation plays an important role in the habituation mechanisms (SHARPLESS and JASPER 1956, CASPERS, LERCHE and GRÜTER 1958). In a recent survey (SCHOLANDER 1961a) it was suggested that the course of a habituation process "is at any moment dependent on the sum of extraneous activating, versus inhibiting impulses streaming into the brain stem reticular formation from other centres in the central nervous system as well as from the different extero- and endoceptors". Thus if a hypothesis might be put forward concerning the neurophysiological background of the high response amplitude peaks observed in the present investigation it could be as follows: The successively increased sleepiness induced by the experimental situation activates feed-back loops between the brain stem reticular formation and, on the one hand, higher nervous centers and, on the other hand, different peripheral sensory mechanisms. These impulses would delay habituation and the sleepiness induced by this process. It is somewhat puzzling to find that the response amplitude peaks seldom appeared simultaneously in the different variables (*cf.* p. 6). However, it is always difficult to compare momentary reactions elicited in different effector systems by a specified stimulus (*cf.* DUREMAN 1959, p. 60, BINDRA 1959, p. 219, SCHOLANDER 1961a, p. 13).

It should be emphasized that at least one critical experiment must be made before the interpretation inherent in the premisses of the present investigation

can be definitely accepted. The results would probably have been quite different if the subjects had been completely relaxed without any "pressure for performance". Under such circumstances the combination of the sleep deprivation and the monotony of the experimental situation might increase the tendency towards both sleep and habituation of the variables as opposed to the results obtained after normal sleep. However, most experimental situations involve some "pressure for performance". It is suggested that the instructions are of critical importance for the effects of sleep deprivation on physiological variables. If no care is taken to promote either complete relaxation or efforts to stay awake some individuals would probably show increased reactivity and delayed habituation. Others would exhibit decreased reactivity and an increased tendency towards both habituation and sleep. Finally, a third category, would be expected to alternate between these response patterns. Inadequate instructions may be one of the reasons why many previous investigators have failed to find any clear physiological changes after sleep loss (*cf.* reviews published by KLEITMAN (1939), TYLER, GOODMAN and ROTHMAN (1947) and BJERNER (1949)). Furthermore it is evident that monotony and prolonged experiments may potentiate the effects of sleep deprivation. It can be mentioned that among a wide variety of psychological tests WILKINSON (1958) found that only two were significantly impaired after a wakefulness of 26—30 hours. Both these tests involved a prolonged monotonous repetition of a simple performance task. It was also shown that the decline of efficiency could be counteracted by giving the subject information of his results. It was concluded that "the less predictable the sleep-less operator finds the task situation and the greater the penalty he suffers for failing to predict it accurately, the less likely is his efficiency to fall below normal levels".

One question which also remains to be answered is whether the present results would have been similar if the period of sleep deprivation had been prolonged. Under such circumstances the conflict between sleepiness and wish to stay awake ought to increase. The effects upon the recorded variables would then be more pronounced. Obviously there are great inter- and intra-individual differences with regard to tolerance of lack of sleep. Above a critical limit the subjects would be unable to withstand the sleepiness induced by the present experimental situation. The effects upon the recorded variables might then be contrary to those obtained in the present study.

It has been a common clinical observation that sleepiness and fatigue if counteracted by will may cause psychosomatic symptoms such as headache, gastro-intestinal complaints etc. Mostly such symptoms have been interpreted as being compensatory phenomena caused by the effort to maintain normal efficiency. Remarkably few experimental data supporting this assumption have been published. The observations made by FREEMAN (1932) indicate that the efforts to keep awake may be accompanied by increased muscular tension. In the present study it has been shown that changes in autonomic response

elements may also occur. However, there is need of further experimentation in this field. Many problems such as those mentioned above remain to be further elucidated.

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Percutaneous Puncture of the Radial Artery with a Multi-Purpose Teflon Catheter for Indwelling Use

By

PER-OLOF BARR

Received 1 December 1960

Abstract

BARR, P.-O. *Percutaneous puncture of the radial artery with a multi-purpose Teflon catheter for indwelling use.* Acta physiol. scand. 1961. *51.* 343—347. — A Teflon catheter assembly is described, which was designed for percutaneous insertion and indwelling use in the radial artery, and in other superficial blood vessels with calibers of a similar order of magnitude. The advantages of the instrument, which may also be used for percutaneous puncture of other superficial tissues and organs, are reported and discussed, especially with regard to the preferential choice of the radial artery for arterial punctures.

Three different methods are currently applied for percutaneous insertion of soft catheters into blood-vessels or other tissues, all of them using the principle of substituting a catheter for the hypodermic needle, which is required for the penetration of skin and tissue: (1) introduction of a catheter through the lumen of the needle (FITZPATRICK, SCHNABEL and PETERSON 1949, PEIRCE 1951, GIDLUND 1956); (2) simultaneous insertion of a catheter and the needle, the needle having previously been pushed into the catheter for support and its protruding tip being used for puncturing (MASSA *et al.* 1950, SELDINGER 1957); and (3) threading a catheter over a flexible leader, the leader having previously been pushed into place through the lumen of the hypodermic needle (SELDINGER 1953, BERNÉUS *et al.* 1954).

While the radial artery is in many instances the vessel of choice for sampling of arterial blood and intravascular blood pressure measurements, *e. g.* when puncturing of deeper arteries may cause dangerous hematoma, none of the needle catheter combinations previously described have proved practical for use with

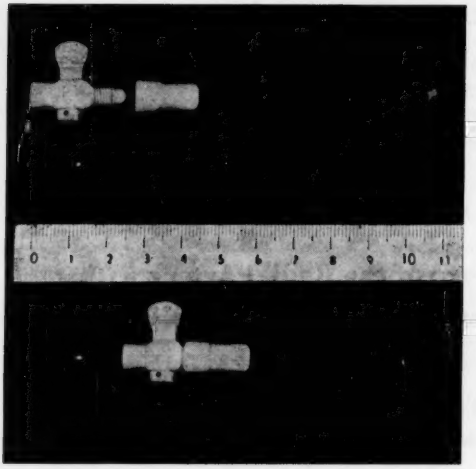

Fig. 1. *Above:* Elements of Teflon catheter. *Below:* The assembled instrument, ready for percutaneous introduction.

Scale in cm.

this vessel. However, after extensive experimentation with plastic tubings of different kinds and dimensions it was found that by using the method of MASSA *et al.* (1950, see above) in combination with thin-walled tubing of Teflon, the radial artery may conveniently be catheterized with a minimum of trauma. While the instrument to be described was primarily designed for this artery, it has proved equally useful for other superficial blood vessels.

Constructional Details

The instrument (Fig. 1) consists of three main parts: 1) the catheter, 2) the "cap and screw" adapter with stopcock, and 3) the puncturing hypodermic needle.

1) The catheter is made of a 50—60 mm long piece of Teflon tubing[1] (inner-diameter 0.80 mm, outer-diameter 1.05 mm). One end of the catheter is heat-moulded into a flange, the other end (tip of catheter) being cut off square and trimmed.

2) The adapter, similar to the metal "cap and screw" types that are commercially available for polyethylene tubing, is made of Teflon and incorporates a stopcock of the same material. To prevent slipping of the catheter, the V-shaped seat inside the "cap" is reinforced by a metal bushing. The flange of the catheter, acting as its own gasket, isolates the metal reinforcement from the lumen of the catheter-adapter.

3) The puncturing needle is a standard hypodermic needle (short-bevel point) with a length of 80—100 mm and an outer-diameter of 0.8 mm.

[1] Tubing supplied by Hammarby Bakelit Industri AB, Stockholm, Sweden.

Technique for Percutaneous Insertion into the Radial Artery

The equipment is assembled by attaching the catheter to the adapter and pushing the needle through the lumen of the catheter-adapter. The needle must fit into the tubing so that it may be gently advanced under rotation. The tip of the steel needle should protrude 2—3 mm out of the trimmed front end of the catheter.

Sterilization can preferably be done in the assembled state by boiling, dry heating or autoclaving. The instrument is inserted into the artery under infiltration anesthesia (for venous punctures anesthesia is not required). In order to facilitate the localization of the radial artery, the penetration of the skin and to reduce the tendency of spastic contractions of the vessel, it is recommended to immerse the wrist and hand in water of 48—50° C for 5—10 min immediately before the insertion. Care should be taken that the anesthetic used is not too concentrated, since the hyperemia greatly increases the rate of absorption and the risk of toxic effects.

Since the diameter of the puncturing segment increases by only 0.25 mm at the tip of the Teflon catheter, very slight extra pressure is needed after the protruding needle tip has been introduced. While the escape of blood from the needle indicates that the needle tip is inside the vessel, the slipping-in of the catheter tip is not always felt by the operator. It is therefore recommended to advance the needle a few millimeters further into the vessel to make sure that the catheter tip has entered. Next, the needle is kept stationary, while the catheter, by applying pressure on the adapter, is slid along the needle further into the vessel (BARR and SOILA 1960). It is essential that the needle is not pulled out until the catheter is well in the vessel. On removal of the needle, blood spurts from the adapter, and the stopcock may now be closed and reopened as needed.

When removing the catheter the same precautions should be observed as when using hypodermic needles for arterial punctures. Thus, immediately after the withdrawal of the catheter, local pressure should be applied, first manually for 5—10 min, then by means of a pressure bandage for 3—4 hours (or more if the subject has been given anticoagulants). Excessive movements of the wrist should be avoided till the following day.

Results

The needle-Teflon catheter combination has been successfully used in this laboratory for almost 2 years in about 200 punctures. Of the insertions performed, about 50 % were made into the radial artery and the rest on various peripheral arteries and veins. Of a total of over 75 subjects, most of them young healthy students, some have had the same radial artery punctured 8—12 times. Several subjects have had catheters in both radial arteries on the same occasion.

Since the opening cut in the artery is small and effective local pressure can easily be applied after removal of the catheter, no serious complications have occurred. In many instances arterial blood was drawn continuously from the catheter directly into a glass-reference-electrode assembly for continuous analysis of arterial blood pH. For these measurements heparine had to be given intravenously in order to avoid clotting in the apparatus (100— 150 mg Heparin Vitrum). In a few of these experiments, after which the subject had removed the bandage against instructions or vigorously moved his wrist before the heparine action had expired, moderate hematoma developed causing slight discomfort for 2—3 days. None of these subjects needed therapy. Permanent after-effects have not been observed.

If handled with care, the same needle, catheter and adapter may, after thorough cleaning, be re-assembled, sterilized, and used repeatedly. However, it is mandatory that the needle is free from the slightest barb, and that the tip of the catheter is checked for accidental deformation.

Discussion

The sampling of arterial blood is imperative in any physiological or clinical laboratory, where investigations aim at complete evaluation of pulmonary gas exchange. Such sampling, as well as intraarterial pressure measurements, require arterial punctures when performed in humans. The present method was developed to eliminate some of the major difficulties and risks currently involved in this procedure. Specifically, a method was needed that could be safely employed for arterial puncture even in subjects treated with anticoagulants.

As to the site of the puncture, the radial artery was preferred, mainly because the risks for accidental obstruction of blood supply to the hand are minimal, the collateral circulation being extensive (cf RADNER 1947). The ease with which effective pressure may be applied over the site of the puncture to counteract any tendency to hematoma is decisive for the choice of this artery when anticoagulants have been given to the subject.

There should be no need to emphasize the advantages of soft catheters for indwelling use in arterial punctures. However, the methods designed for percutaneous insertion of flexible catheters into vessels (see above) were not found suitable for the radial artery either because the desired catheter lumen required a disproportionately big hole in the vessel wall, or because the needle-catheter combination could not easily be heat-sterilized.

While the instrument of MASSA *et al.* (1950) exhibits the above-mentioned drawbacks, the method otherwise offers a most elegant solution to the problem. A search among various plastics (BARR and SOILA 1960) showed that fluorocarbons might be used as catheter material to eliminate the disadvantages mentioned.

Mechanically, Teflon tubing proved excellent for use with the relatively narrow radial artery, since the thickness of the material could be reduced by more than 50 per cent, the tubing still retaining a sufficient strength to follow the needle tip through the skin, tissues and arterial wall without becoming deformed. The low coefficient of friction is another advantage of this material when used for introduction into tissues by the present procedure. Furthermore the tubing shows little tendency to kinking until the radius of bending becomes less than about 6 mm.

Chemically, Teflon is the most inert plastic and the one least apt to cause tissue reaction (Le Veen and Barberio 1949, Harrison *et al.* 1957). Since the wettability of Teflon is practically nil, this plastic is less prone to induce clotting than most other materials.

Finally, the thermal stability of Teflon allows sterilization of the entire catheter assembly by boiling or dry-heating up to 200° C, whereby infection due to imperfect "sterilization" by germicide, generally used for less heat-resistant plastics, can be avoided.

Even if the present needle-Teflon catheter combination was primarily designed for the radial artery, it has proved equally useful and convenient for other superficial arteries and veins, and should also be suitable for the percutaneous puncture of organs other than blood vessels.

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Improved Technique for the Fluorimetric Estimation of Catecholamines

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Abstract

Euler, U. S. v. and F. Lishajko. *Improved technique for the fluorimetric estimation of catecholamines.* Acta physiol. scand. 1961. *51.* 348—356. — By the addition of small amounts of ethylene diamine (EDA) to the alkali-ascorbic acid mixture used in the trihydroxyindole (THI) method the discoloration of reaction mixture and instability of fluorescence can be prevented, allowing blanks to maintain their fluorescence values for several hours. The lutines obtained from adrenaline and noradrenaline standards and from alumina eluates are stable over a period of at least one hour. Optimal fluorescence values are obtained when the volume of alkali-ascorbic-acid — EDA exceeds the volume of the sample by 1 ml. A proportional increase in fluorescence is observed with sample volumes up to 3 ml. The technique used for estimation of catecholamines in urine and organ extracts is described. The importance of the acidity for the release of catecholamines from conjugates in urine at room temperature is emphasized.

Adrenaline and noradrenaline can be conveniently and accurately estimated in small amounts by fluorimetric technique using the trihydroxyindole (THI) method of Ehrlén (1948) and Lund (1949). When occurring in a mixture the two amines can be determined differentially, after transformation to lutines, by the use of two filter sets (Price and Price 1957, Cohen and Goldenberg 1957, Euler and Lishajko 1959) or by using spectrophotofluorimetric technique (Bertler, Carlsson and Rosengren 1958, Vendsalu 1960).

Stabilization of the lutines is achieved by addition of ascorbic acid (Ehrlén 1948, Lund 1949). It is observed, however, that the mixture of ascorbic acid and strong alkali tends to age rapidly, forming red-coloured compounds. This change is accompanied by an increase in the blank values and, under certain conditions, a decrease of the net fluorescence with time. For this reason

readings have to be made within a relatively short period after the reaction has been carried out, when extracts are used which have previously been purified by passage through an alumina column and eluted with acetic acid.

The present report deals with some observations made on the effect of addition of certain compounds on the stability of the reagent mixture and of the lutines formed from adrenaline and noradrenaline.

Experimental

1. *Stabilization of reagent blanks*

Spontaneous appearance of a reddish tint usually occurs in the alkali-ascorbic acid mixture after some 7—10 min and develops further in the course of 1/2 hour. During the same time, the initially slight fluorescence increases in the alkali-ascorbic acid mixture, rapidly during the first 5—10 min and then more slowly, reaching a maximum in 15—30 min after which it remains unchanged over 4—6 hours. This increase in fluorescence is small for filter set A according to COHEN and GOLDENBERG (1957), consisting of primary interference filter 395—415 mμ and secondary filter Ilford Bright 623, peak transmission 490 mμ, but is considerable for filter set B, consisting of primary interference filter 436 mμ and secondary filter Corning 3486 with peak transmission 540 mμ. On addition of potassium ferricyanide the development of the fluorescence is markedly enhanced, particularly for filter set B.

It has been observed, however, (F. L.) that the increase in fluorescence is strongly inhibited by addition of small amounts of ethylene diamine (EDA) to the alkali-ascorbic acid mixture. No change of fluorescence with time was observed by addition of ferricyanide to alkali alone or to ascorbic acid alone. Both ferricyanide and EDA caused, however, a slight but constant increase in the blank figures.

By addition of ethylene diamine the development of the red colour in the alkali-ascorbic acid mixture was also postponed so that a slight reddish tint was observed only after 3—4 hours.

Effect of EDA on fluorescence of blanks with standard solutions of adrenaline and noradrenaline

Faded blanks were prepared by adding to 3 ml of the samples, containing 0.5 μg of adrenaline or noradrenaline, 0.1 ml 0.25 per cent potassium ferricyanide and 2.7 ml 20 per cent sodium hydroxide. After 3 min 0.3 ml 2 per cent ascorbic acid was added.

Non-faded blanks were obtained by addition of 3 ml alkali-ascorbic acid mixture + 0.1 ml 0.25 per cent potassium ferricyanide to 3 ml of the sample containing 0.5 μg of adrenaline or noradrenaline standard respectively.

Without addition of EDA the fluorescence of both the faded and the non-faded blanks with adrenaline, as well as noradrenaline, increased, for filter set A by about 50 per cent and for filter set B by as much as 6 times in 60 min.

Table I. Fluorescence (scale divisions) of blanks with standard solutions of A and NA. Faded and non-faded blanks with and without EDA

Standard	Blank	5 min		25 min		60 min	
		A	B	A	B	A	B
Noradr. 0.5 µg	Faded	3.5	2.0	3.6	12	5.2	11.7
»	Faded + EDA....	3.0	1.5	3.1	2.5	3.3	2.4
»	Non-faded.....	3.0	1.7	4.5	12	5.4	11.0
»	Non-faded + EDA	3.5	2.0	3.3	2.3	3.5	2.2
Adr. 0.5 µg	Faded	3.0	2.0	3.9	13.7	5.3	12.4
»	Faded + EDA....	3.2	1.7	3.2	2.3	3.3	2.2
»	Non-faded.....	3.2	2.0	4.7	11.8	5.0	10.5
»	Non-faded + EDA	3.8	3.4	3.9	4.0	4.0	4.0

When 0.2 ml EDA were added to 10 ml alkali-ascorbic acid mixture no increase in the sample blanks was observed over a period of 60 min (Table I).

Adrenaline and noradrenaline standard blanks prepared each hour over 4 hours with the same alkali-ascorbic acid mixture to which EDA had been added (2.5 : 100) showed no increase in the fluorescence values.

The minimum amounts of EDA required to prevent the development of the red colour in the reaction mixture, and to keep the reagent and sample blanks low, were found to be about 0.2 ml per 10 ml alkali-ascorbic acid mixture. Addition of 0.05—0.1 ml EDA to 10 ml had a marked although not complete protective action, while 0.01 ml was clearly insufficient. Since no advantage was observed by using higher amounts of EDA, which on the contrary tended to diminish the readings, the addition of 0.2 ml EDA per 10 ml alkali-ascorbic acid solution was used as standard procedure, EDA being mixed with the alkali before addition of the ascorbic acid.

Effect of EDA on fluorescence of standard solutions of adrenaline and noradrenaline

The net fluorescence values of standard solutions of noradrenaline and adrenaline after transformation to the corresponding lutines with an alkali-ascorbic acid mixture were well maintained for 1 hour with and without EDA (Table II) and thereafter declined slowly.

As seen in the table the net fluorescence values for noradrenaline were markedly higher if transformation to lutines was made with alkali-ascorbic acid to which EDA had been added. This occurred even if the alkali-ascorbic acid solution containing EDA was 4 hours old. The net fluorescence values for adrenaline did not change, however, after addition of EDA. Even after 4 1/2 hours with the sample at room temperature and without protection against day-light the decrease was less than 10 per cent. For adrenaline a decrease in fluorescence of about 5 per cent in 1 hour was observed in filter set B whether or not EDA was added.

Table II. Blanks (Bl.) and net fluorescence (Net Fl.) of lutines from adrenaline (0.25 μg) and noradrenaline (0.5 μg) standard. Alkali-ascorbic acid mixture alone or with EDA prepared at time 0. Non-faded blanks. Filter sets A and B

		Adrenaline				Noradrenaline			
		Bl.	Net Fl.	+ EDA		Bl.	Net Fl.	+ EDA	
				Bl.	Net Fl.			Bl.	Net Fl.
5 min	A.....	3.0	11.3	3.0	11.2	3.3	25.2	3.2	32.1
	B.....	4.8	33.2	2.2	33.3	4.0	24.6	3.4	29.1
60 min	A.....	4.0	11.0	3.2	11.1	4.7	26.4	1.8	32.2
	B.....	10	31.4	2.3	31.5	8.9	25.2	2.2	28.6

The stabilizing effect of EDA was observed in the same way with 3 ml samples of a solution of 8 ml N HCl + 2 ml 1 M Na-acetate buffer pH 6.4 and containing 2 μg noradrenaline. The solution corresponds to the eluate obtained from ion exchange resins in the technique described by BERTLER *et al.* (1958).

Eluates from urine and from organ extracts after adsorption on alumina

Normal urine was adsorbed on an alumina column and eluted according to the technique of EULER and LISHAJKO (1959). Fig. 1 shows the fluorescence values for blanks and the net values for samples with 3 ml eluate and 3 ml alkali-ascorbic acid, with and without EDA. It can be seen in the figure that the net fluorescence values are moderately increased after addition of EDA, as in the case of noradrenaline standard. Without the addition of EDA the net fluorescence gradually declines over a 1 1/2 hour period, while no such decrease is observed after addition of EDA.

A number of tests were made with alumina eluates from trichloracetic acid extracts of heart, spleen and liver of guinea-pig. In all instances the fluorescence figures in samples and blanks were constant over a period of 1 hour, when EDA was added to the alkali-ascorbic acid mixture.

Stabilizing effect of other compounds

The marked stabilizing effect of EDA on the development of fluorescence in the reagent mixture used in the THI-method made it appear of interest to study the action of some related compounds in this respect. The following compounds were tested: propylene diamine, pentamethylene diamine, methylamine, ethylamine, sodium sulphide, hydroxylamine, glutathion.

Propylendiamine added to a concentration of 20 mg/ml in the alkali-ascorbic acid mixture and hydroxylamine 0.1—0.3 mg/ml offered almost as good a protection as ethylenediamine in the same concentration. Several of the other compounds also had a stabilizing action as shown by a delayed

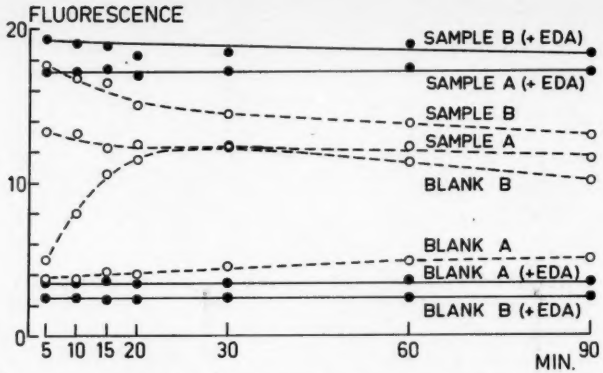

Fig. 1. Fluorescence values of blanks and samples from alumina eluates (urine) with and without addition of ethylene diamine (EDA) to the alkali-ascorbic acid. A and B denote filter sets. Fluorescence of samples are net values.

onset of coloration and maintenance of blank-values. None of them appeared to offer advantages when compared with ethylene diamine, however.

Transformation of chromes to lutines

In a previous communication (EULER and LISHAJKO 1959) it was stated that the amount of alkali-ascorbic acid mixture which had to be added to the sample in order to obtain maximal formation of lutines depended on the volume of the samples. Thus it was recommended that for each ml of the sample 1 ml of the alkali-ascorbic acid should be added in order to obtain maximal fluorescence.

In the present study similar tests were performed, with EDA added to the alkali-ascorbic acid mixture.

Sample volumes of 0.5—5 ml of urine eluates were used and the amount of alkali-ascorbic acid containing EDA for optimal fluorescence determined. In order to reach maximal net fluorescence it was found that the same volume of alkali-ascorbic acid plus 1 ml was necessary as a rule in the range of 0.5—3 ml sample. With 4 ml of the sample the fluorescence was 5—10 per cent less than proportional when compared with the results for 1—3 ml. It was not possible to obtain an approximately proportionate fluorescence within the volume limit of 10 ml when 5 ml eluate was used. For this reason it is recommended that the volume of the eluate used should not exceed 3 ml. Good proportionality of the fluorescence values was found up to this limit. With a 2 ml sample of an alumina eluate from urine, the net fluorescence in per cent of the maximum with filter set A was 75, 84, 96 and 100 % when 0.5, 1, 2, and 3 ml respectively of the alkali-ascorbic acid — EDA mixture were used. With filter set B the corresponding figures were 70, 82, 97, and 99 %.

The amount of ascorbic acid necessary to give optimal fluorescence was 1 ml of a 2 per cent solution in 9 ml 20 per cent Na OH to which 0.2 ml EDA had been added.

Oxidation time

Although 2 min were an adequate oxidation time for standards it was found that urine eluates required 3 min oxidation time in order to give optimal fluorescence values in several test series. Further increase in oxidation time did not increase the fluorescence figures.

Routine procedure for urine and organ extracts

To the filtered urine sample, usually 25 ml, 0.5 g of the disodium salt of ethylene diamine tetraacetic acid (EDTA) is added, and pH adjusted to 8.2—8.5 with N sodium hydroxide, added dropwise under continuous agitation with a magnetic stirrer and controlled by a glass electrode. The urine is then immediately passed through a column of 10 mm diameter containing 1 g aluminium oxide (British Drug Houses). The aluminium oxide is previously swirled around in distilled water and the resulting suspension of very fine particles, which may clog the glass filter of the column, decanted. This procedure is repeated 2 or 3 times or until the alumina settles rapidly, leaving a clear aqueous layer.

The 15 cm high glass tube is fitted with an upper wider part to hold 50—100 ml and a glass filter D1 and a glass stopcock in the lower part. The total passage time for the urine filtrate should not exceed 15 min. If passage tends to become slow, air pressure can be applied to the column vessel so as to maintain a flow of at least 5 ml per min. Smaller volumes of urine than 25 ml are conveniently diluted to this volume with glass-distilled water. After passage of the urine the glass walls and the column are thoroughly sprayed and washed with bi-distilled water until the washing fluid is completely free from alkali, which is essential.

Elution is performed with 0.25 N acetic acid of which 5 ml is thoroughly mixed with the alumina and allowed to filter off. Thereafter the same volume of acetic acid is added. The eluate is centrifuged at 600—1,000 *g* for a few minutes in order to sediment fine alumina particles that may have passed through the filter.

In the eluate, which should have a pH value of about 3.5 and should not exceed pH 4.0, the catecholamines are stable at room temperature for several hours. When kept frozen, the eluate can be used for analyses weeks later. Immediately before analysis, the eluate is adjusted to pH 6.2—6.3 with 2 N ammonia which is added dropwise under stirring with continuous control by the glass electrode. The eluate at pH 6.2—6.3 should be used for analysis as soon as possible in order to prevent spontaneous oxidation. Usually no losses are observed within 20 min, however.

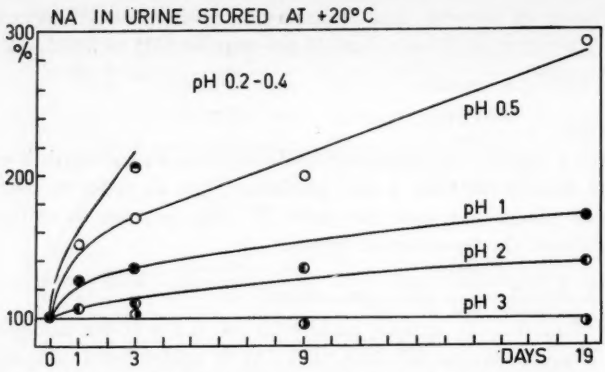

Fig. 2. Changes in free noradrenaline in urine kept at room temperature at pH 0.2—3 for various lengths of time. The curves represent different urines.

For larger volumes of urine a wider column may be used with a layer of alumina 1—2 cm high, allowing adsorption to be completed in 15 min. Elution is then made with acetic acid of sufficient volume and strength so as to maintain a pH value of 3.5—4 in the eluate.

Tissues and organs are minced and extracted with 2—5 volumes 10 per cent trichloroacetic acid for 1/2 hour, filtered on a suction funnel and washed with 5 per cent trichloroacetic acid. The combined extracts are adjusted to pH 8.2—8.3 with NaOH.

To n ml of the sample, pH 6.2—6.3, in a graduated cylinder, is added 0.1 ml 0.25 per cent potassium ferricyanide. Oxidation is carried out during 3 min. After this time n + 1 ml alkali-ascorbic acid containing 0.2 ml EDA per 10 ml is added and glass-distilled water filled up to 10 ml. After thorough mixing, the fluorescence is read in a fluorimeter with filter sets A and B. The Coleman 12 C fluorimeter has proven suitable for estimations in urine and most organ extracts. The blanks are prepared by adding alkali-ascorbic acid — EDA to the sample and then ferricyanide.

Influence of pH on free catecholamines in urine

It is well known that when urine is heated at acid reaction catecholamines are released from readily hydrolyzable conjugates. In order to ascertain the pH limit at which urine could be kept at room temperature without change in the proportion of free catecholamines, urines were adjusted to pH-values 0.2—3 and samples analyzed after various lengths of time. As seen in Fig. 2 considerable increases in free noradrenaline are observed at pH-values below 1. At pH 3 the content of free catecholamines in urine shows insignificant changes over periods of more than 2 weeks. The results demonstrate the importance of maintaining an adequate reaction in urine stored before analysis.

Discussion

Although the trihydroxyindole (THI) method for the fluorimetric estimation of adrenaline and noradrenaline may be regarded as a simple and reasonably accurate method, the stability of the fluorescence in blanks and samples is often unsatisfactory. Thus the fluorescence of the blanks begins to increase in a few minutes, particularly when measured with filter set B according to COHEN and GOLDENBERG (1957). This does not invalidate the net fluorescence values of standards to any marked degree, since these increase approximately by the same number of scale divisions. This is true also for an alkali-ascorbic acid mixture kept at room temperature for several hours. Since the blank values increase gradually for about 15 min the values may be inconvenient for the reading of low net fluorescence, however.

By the addition of a small amount of ethylenediamine to the alkali-ascorbic acid mixture it has been possible to stabilize fluorescence over a period of several hours. It thus becomes unnecessary to prepare new alkali-EDA-ascorbic acid mixture at frequent intervals. Care must be taken, however, to minimize the uptake of carbon dioxide from the air.

In addition to the advantage of maintaining low blank values another even more important advantage has been achieved. As mentioned above the fluorescence values for standard solutions show only little change over 1 hour, but with samples prepared from urine, after adsorption on alumina and elution with acetic acid, the fluorescence shows a gradual decline, particularly of the values obtained with the filter B set. With the addition of EDA the fluorescence values obtained with such samples were constant for periods exceeding 1 hour. The moderate increase in fluorescence observed with filter sets A and B for noradrenaline is another advantage.

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Localization of Focal Potentials Evoked in the Red Nucleus and the Ventrolateral Nucleus of the Thalamus by Electrical Stimulation of the Cerebellar Nuclei

By

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Abstract

Appelberg, B. *Localization of focal potentials evoked in the red nucleus and in the ventrolateral nucleus of the thalamus by electrical stimulation of the cerebellar nuclei.* Acta physiol. scand. 1961. *51.* 356—370. — The experiments described were performed on cats anesthetized with Nembutal. Electrical stimulation was applied to the cerebellar nuclei with stereotactically guided stimulating electrodes. Focal potentials evoked in the mesencephalon and in the thalamus were recorded with glass or tungsten microelectrodes. The potentials were histologically localized to the red nucleus and the ventrolateral thalamic nucleus. It was found that monosynaptic pathways exist between the interposite nucleus in the cerebellum and the red nucleus on one hand and between the interposite nucleus and the ventrolateral nucleus of the thalamus on the other. No activation of the investigated regions could be obtained by stimulation of the dentate or fastigial cerebellar nuclei.

It is well known that the red nucleus receives its main afferent supply from the contralateral deep cerebellar nuclei (Mussen 1927, Papez and Stotler 1940, Carrea and Mettler 1954, Carpenter and Stevens 1957). It is also known that some of the fibres of the brachium conjunctivum pass through the red nucleus without relaying. According to Allen (1924), Mussen (1927), Carrea and Mettler (1954) these fibres end in the ventrolateral nucleus of the thalamus. For many years the opinion seems to have been that both the interposite and the dentate nuclei are the cerebellar source for fibres to

the red nucleus. On the other hand JANSEN and JANSEN (1955) came to the conclusion that the dentate nucleus is mainly related to thalamocortical systems, while the interposite nucleus is associated with midbrain structures.

The present investigation was undertaken in order to reveal whether electrical stimulation in the cerebellar nuclei evoked a focal potential in the red nucleus. If this was the case, it would be the aim to find out if such a focal potential was localized to the red nucleus only, thereby separating this structure from the rest of the mesencephalic reticular formation. This was regarded as being of importance for future electrophysiological work on the red nucleus and its relationships. During the course of the experiments it also became necessary to find the origin of the rubral fibres in the cerebellar nuclei and to localize a thalamic response evoked simultaneously with that observed in the red nucleus.

Materials and methods

The results presented were derived from 38 cats. They were anesthetized with Nembutal, the initial dose (25 mg/kg) administered intraperitoneally and repeated doses of 5—10 mg given intravenously when needed. The operation consisted in exposing the external meati accustici by sectioning the ears near the skull and in making trephine holes for the microelectrode to the mesencephalon and/or the thalamus, and for the stimulating electrode to the cerebellar nuclei. The animal was mounted in a Horsley-Clarke frame which carried the holder for the stimulating electrode. This holder was inclined 15° from the vertical plane, thereby orienting the stimulating electrode approximately in parallel to the bony tentorium.

The microelectrodes were guided with the aid of a micromanipulator of the type designed by G. J. Winsbury and described by ECCLES *et al.* (1954).

For stimulation in the cerebellar nuclei a mediolateral row of 5 steel needle electrodes insulated to about 0.4 mm from the tip and placed 1 mm apart was used. Stimulation was performed between pairs of nearby electrodes in the row. In some cases a single concentric electrode with an interpolar distance of about 0.5 mm was used instead. The site of stimulation was marked by electrocoagulation.

Recording was made with NaCl-filled glass pipettes, usually with rather crude tips (around 2—5 μ). These were able to record a focal potential which was not distorted by single unit activity. A chlorided silver plate screwed in bone over the frontal sinus was used as a reference.

In some experiments tungsten electrodes were used instead. These electrodes were prepared according to a method modified from HUBEL (1957). The tungsten wire (diameter 0.125 mm) was drawn into a glass tube with the microelectrode puller used for making the ordinary glass pipette electrodes. About 5 mm free tip of the wire was then sharpened electrolytically according to the Hubel technique down to a tip diameter around 1 μ. The whole electrode except the very tip was then covered by an insulating laquer (Voltalack 435, Standard Varnish Works Nordiska Aktiebolag, Göteborg) by lowering it tip upwards into a tube with thin laquer. Baking in 180° three times gave a thin but very resistant coating up to the tip.

These electrodes were well suited for recording from structures lying deep under the cortical surface as they could be made long and slender but still rigid thanks to the glass covering. They had the advantage that recording sites could be marked by means of electrolytic lesions. These lesions were made by passing a direct current of 5 μA for 10 sec through the tip of the electrode (tip negative).

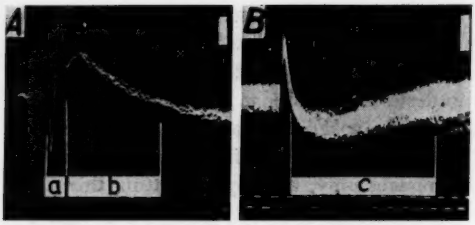

Fig. 1. A. Focal potentials in the red nucleus evoked by electrical stimulation of the cerebellar nuclei. Time constant (Tc) 50 msec. Voltage calibration 100 μV. Time 1 msec.

B. Same as A but slower sweep speed. Tc 0.5 sec. Time 10 msec.

For explanation of letters a, b and c see text.

For microelectrode recordings an amplifier with a time constant which could be varied from 5 msec to 90 sec was used. As a rule time constants of 50 to 500 msec were used. Slow potentials of low amplitude could be checked by switching the amplifier over to DC recording. The potentials were displayed on a double beam CRO and recorded on 35 mm film with a Grass camera. In all records negativity is recorded upwards.

A Grass stimulator (S4) and a stimulus isolation unit (SIU4) were used for electrical stimulation. They were connected to a stimulus distribution unit which permitted rapid connection to any one of five output receptacles.

After each successful experiment the head of the animal was perfused with Ringer's solution and with Bodian's fluid. The brain was then embedded in celloidin. Sagittal or transverse serial sections, 50 or 100 μ thick were made through the cerebellum and through the mesencephalon and the thalamus. The sections were stained with toluidin blue (Nissl). As a rule the tracks made with the stimulating electrode as well as with the microelectrodes could be seen and identified on the sections. Electrolytic lesions and electrocoagulations were always readily visible.

Results

I. *Focal potentials evoked in the mesencephalon and in the thalamus by electrical stimulation of the cerebellar nuclei*

Electrical stimulation within the cerebellar nuclear complex evoked a characteristic focal potential in the mesencephalon in the region of the red nucleus. It consisted of an initial positive-negative spike (a in Fig. 1 A) of short latency (mean latency to the maximum of the positivity 0.49 msec, SD 0.09). This spike was followed by a negative wave (b in Fig. 1 A) with a mean latency of 1.25 msec (SD 0.19). Its duration ranged between 2 and 6 msec. As a rule the potential complex ended with a longlasting positive deflection the duration of which varied between 50 and 100 msec (c in Fig. 1 B).

If a synapse blocking agent (Myanesin) was administered intravenously while recording a response of the type described above the negative wave (b in Fig. 1 A) and the shallow positivity (c in Fig. 1 B) were seen to diminish and finally to disappear although the initial positive-negative spike (a in Fig. 1 A) remained relatively undisturbed (as shown in Fig. 2 C). A deepening of the anesthesia had a similar effect as is seen to the right in Fig. 2. Also

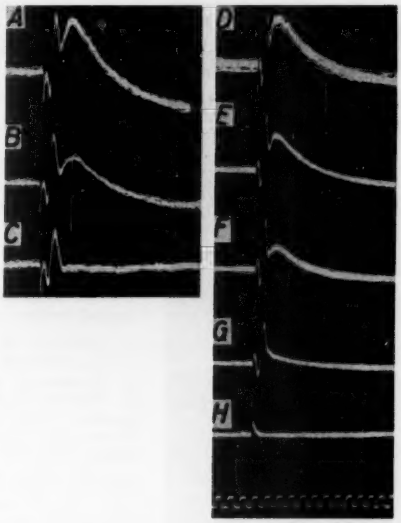

Fig. 2. To the left:

A. Control record of focal potentials in the red nucleus on cerebellar stimulation.
B. 2 min after i.v. injection of Myanesin (75 mg/kg).
C. About 50 sec later.

To the right:

D. Control record of potentials in the red nucleus.
E. After a total amount of 80 mg Nembutal given in 20 mg doses during 13 min.
F. After a total of 175 mg in 33 min.
G. After a total of 290 mg in 50 min.
H. Cat dead for about 10 min. Shows shape of stimulating artifact.

Tc 50 msec. Time 1 msec.

severe anoxia produced by stopping the artificial respiration in a curarized preparation made the negative wave disappear before the initial spike.

The type of response shown in Fig. 1 closely resembles that described by Brooks and Eccles (1947) and by Eccles *et al.* (1954) for synapses in the spinal cord. It seems reasonable to interpret the different components of this focal potential in accordance with the above mentioned authors. Thus the initial positive-negative spike is attributed to the presynaptic volley and the slow negative wave is regarded as beeing of postsynaptic origin. Such an interpretation is supported by the results of Myanesin administration, increase in depth of anaesthesia, and anoxia.

The postsynaptic potential was found only within a very restricted area, while the presynaptic spike was more widely spread. When appearing alone, this spike was seen to be triphasic, positive-negative-positive. It was always considerably smaller in amplitude when recorded laterally, medially, dorsally and ventrally to the area giving a full response. Caudally to this area, between the red nucleus and the cerebellum, the presynaptic spike was big, and in a rostral direction it could be traced relatively unchanged up to the thalamus. There it was once more followed by a postsynaptic negative wave. Fig. 3 presents the shape of the presynaptic spike caudally and rostrally to the red nucleus (A and C) and also in this nucleus (B) and in the thalamic responding region (D). As shown in the figure the latency to the presynaptic spike increased in a rostral direction. The latency to the maximum of the initial positivity

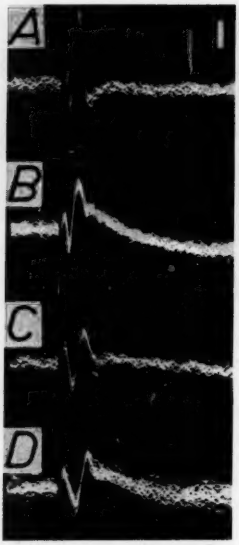

Fig. 3. Response to cerebellar stimulation at the following frontal levels:

A. Caudally to the red nucleus. Latency to maximum of initial positivity 0.41 msec.
B. In the red nucleus. Latency 0.50 msec.
C. Halfway between the red nucleus and the ventrolateral nucleus of the thalamus. Latency 0.63 msec.
D. In the ventrolateral nucleus of the thalamus. Latency 0.83 msec.

Distance from the level of the cerebellar nuclei is to A about 7 mm, to B about 9 mm, to C about 11.5 mm and to D about 16 mm. Tc 50 msec. Voltage calibration 100 μV. Time 1 msec.

was thus about 0.45 msec caudally to the red nucleus (Fig. 3 A) and about 0.85 msec in the thalamus (Fig. 3 D).

During the experimental work at the thalamic level it became evident that the latencies of the different components were not the same in the whole responding area. During penetration of a microelectrode through the thalamus a response sequence of the type illustrated in Fig. 4 was regularly seen. Thus, the first sign of a response was a small, rather badly defined presynaptic potential followed by a post-synaptic negative wave with a latency of about 2.3 to 2.5 msec (Fig. 4 A). As the microelectrode was pushed ventrally the postsynaptic potential suddenly diminished in amplitude while the presynaptic spike grew more visible (Fig. 4 B). A little deeper the negative wave indicating synaptic activity once more increased. This wave, however, now had a latency varying between 1.5 and 1.8 msec (Fig. 4 C). This type of response was then seen through the rest of the responding part of the track. The latency difference of about 0.7 msec makes it probable that the dorsally recorded response is mediated from the cerebellum via a path with one extra synapse.

II. *The localization of the focal potentials to morphological structures within the mesencephalon and the thalamus*

In these experiments the recording microelectrode was placed in a position which according to Horsley-Clarke coordinates ought to be the red nucleus or the ventrolateral nucleus of the thalamus. Then the stimulating electrode

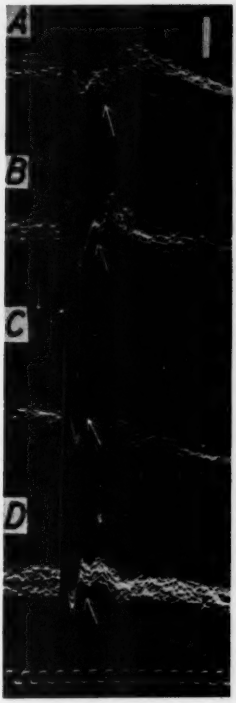

Fig. 4. Response obtained from a microelectrode in the ventrolateral nucleus of the thalamus. Electrical stimulation in the cerebellar nuclei.

A. First sign of evoked potential. Latency to postsynaptic wave (arrow) 2.50 msec.
B. 1 mm more ventrally. Weak response of the same type. Latency 2.40 msec.
C. 0.50 mm further ventrally. Strong response with latency to negative wave 1.60 msec.
D. Weak response of same type near the ventral border of response (3 mm ventrally to C).

Tc 50 msec. Voltage calibration 100 μV. Time 1 msec.

was guided to a position in the cerebellar nuclei where electrical stimulation evoked a good response from the microelectrode. The stimulating electrode was kept at this point throughout the rest of the experiment. The stimulating intensity was usually between two and three times that evoking a threshold response.

Rows of microelectrode tracks 0.5 to 1.0 mm apart were then made through the responding regions. Usually only one or two such rows were made in one experiment. In each track photographic recordings were made at regular intervals (0.25 or 0.50 mm).

The tracks were then identified on serial sagittal or frontal histological sections. Tracings were made from enlarged sections with identified tracks. As a rule, all tracks in a grid were not found in one and the same section. A diagram was therefore made presenting all tracks on a tracing of a representative histological section. This procedure was generally satisfactory, as the borders of the nuclei did not differ considerably between nearby sections. In some cases, however, an error was introduced. This is then commented upon in the text or indicated on the diagram.

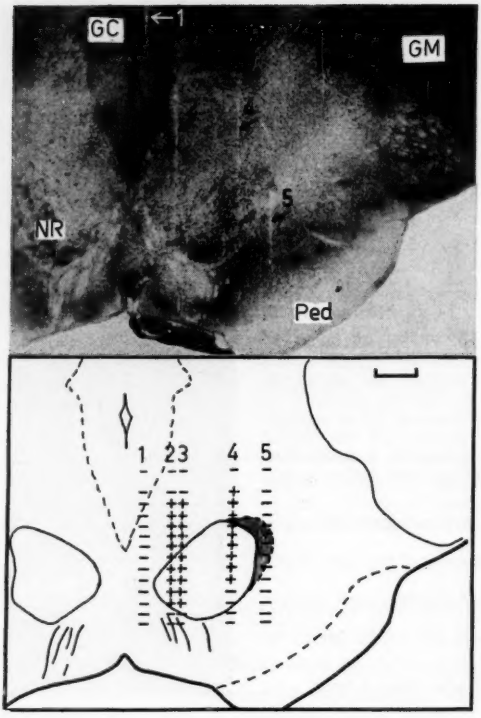

Fig. 5. Frontal section through the mesencephalon at the level of the red nucleus (top) and diagram of the same section (below). In this and the following figures faint traces of tracks are indicated by numbered arrows on the sections. Corresponding numbers are found in the diagrams. Plus symbols (+) on the diagrams indicate response to cerebellar stimulation and minus symbols (—) indicate lack of such response. GC, griseum centrale. GM, corpus geniculatum mediale. NR, nucleus ruber. Ped, pedunculus cerebri. Scale 1 mm.

The localization of the response in the mediolateral and rostrocaudal direction was established by the identification of the microelectrode tracks in the histological slide. The determination of a recording point along a track, *i. e.* the dorsoventral localization was, however, subject to some error introduced by the Horsley-Clarke technique. It was due to the inaccuracy of using the earplug center as a reference point. Tungsten electrodes were utilized in order to determine the localization of a recording site in the dorsoventral direction. With these electrodes it was possible to make electrolytic lesions, thereby marking the site of the electrode at the moment it was recording a response.

Fig. 5 shows a frontal section through the mesencephalon at the level of the red nucleus. Four microelectrode tracks (marked by arrows and numbered 1, 2, 4 and 5) can be seen. On the diagram below, which is a tracing of the same section, the tracks are shown in their whole length. Track 3 in the diagram was identified on a nearby section. Plus symbols in the diagram indicate a response evoked by electrical stimulation in the cerebellar nuclei. Minus symbols indicate lack of such response. It becomes evident that the

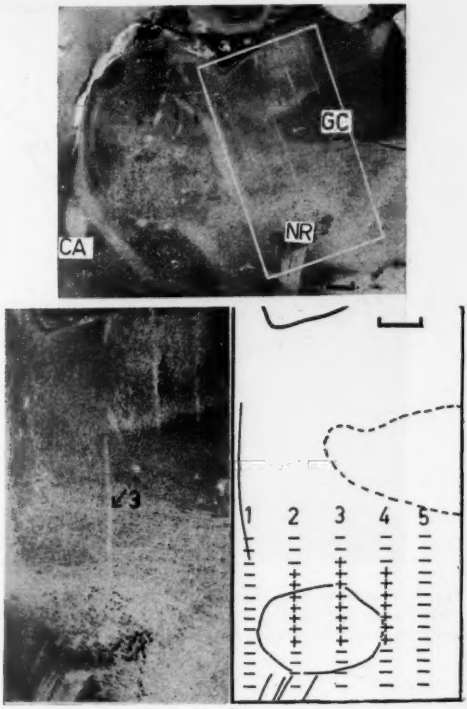

Fig. 6. Sagittal section through the brainstem about 3 mm laterally to the midline (top). The framed area is shown below to the left in higher magnification. Below to the right is a diagram of the same area. GC, griseum centrale. NR, nucleus ruber. CA, commissura anterior. Scale 1 mm.

response was seen only in the tracks passing through the red nucleus and was not observed either medially or laterally to that structure. It should, however, be noted that track 5 in Fig. 5 did not show a response although it is actually seen to pass through the lateral part of the red nucleus. A careful examination of the section reveals that the lateral part of the nucleus here consists of a well defined pool of very small cells (the shaded area medial to the broken line). This is probably the so called nucleus minimus described by MAHAIM (1894) and by VON MONAKOW (1909).

The rostrocaudal extent of the mesencephalic response is shown in Fig. 6. In the upper part of this figure a sagittal section through the brain stem approximately 3 mm laterally to the midline is shown. The framed area is seen in higher magnification below to the left. Three tracks numbered 3, 4 and 5 are seen in the section. The diagram shows five tracks, of which tracks 1 and 2 were identified on nearby sections. Tracks passing rostrally and caudally to the red nucleus were negative. The ventral part of track 4, which appeared about 200 μ medial to the traced section, was seen to pass through the caudalmost cells of the red nucleus.

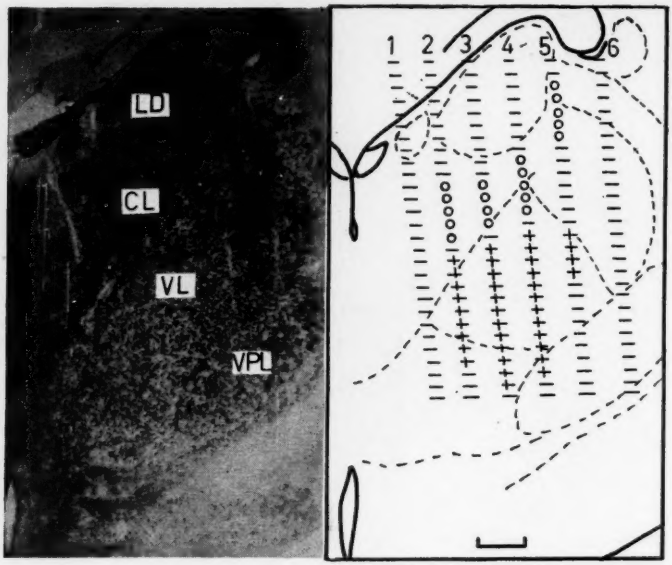

Fig. 7. Frontal section through the thalamus at the level of the ventrolateral nucleus (to the left). To the right a diagram of the same section. LD, nucleus lateralis dorsalis. VPL, nucleus ventralis postero-lateralis. Scale 1 mm.

The disagreement between the borders of the red nucleus and the dorso-ventral extent of the responses seen in Fig. 5 and 6 is due to the errors introduced by the Horsley-Clarke technique. Such errors were, however, as previously described overcome by the use of tungsten electrodes. An experiment of this type is illustrated in Fig. 9 A. This figure shows a frontal section through the red nucleus. A part of a track made by a tungsten microelectrode is seen passing through the red nucleus. An electrolytic lesion was made in a position which yielded typical pre- and postsynaptic waves of the type earlier described. The lesion (at the tip of the arrow) is seen to be situated within the red nucleus.

The localization of the thalamic response is shown in Fig. 7, 8 and 9 B, C and D. Histological sections with identified tracks are given as well as diagrams drawn from these sections and showing all the tracks made in transversal and longitudinal grids through the region of the ventrolateral nucleus. Tracks not found in the histological sections presented were identified in nearby sections. The o-symbols in the figures indicate a long latency response of the type earlier described (Fig. 4 A). In Fig. 7 with a mediolateral row of tracks through the thalamus good agreement is found between the responding tracks and the tracks passing through the ventrolateral nucleus. Fig. 8 with a similar

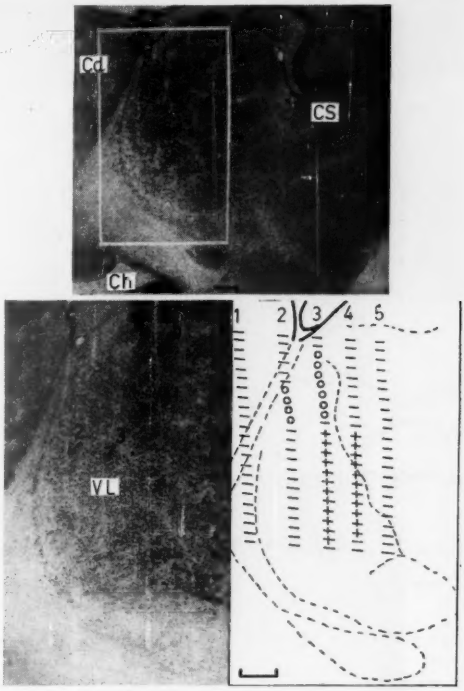

Fig. 8. Sagittal section through the brain stem about 5 mm laterally to the midline (top). The framed area is shown below to the left in higher magnification. Below to the right is a diagram of the same area. Cd, nucleus caudatus. CS, colliculus superior. VL, nucleus ventralis lateralis. Ch, chiasma opticum.

grid in the rostrocaudal direction is more difficult to evaluate. The rostral border of the ventrolateral nucleus is obscure. Therefore it is questionable whether track number 2 passes through, or rostrally to, the nucleus. Tracks 3 and 4, however, no doubt fell within the borders of the ventrolateral nucleus. In the experiment illustrated by Fig. 9 B an electrolytic lesion was made at a point where a typical thalamic response of the type with short latency was recorded. The lesion is seen to be centrally placed in the ventrolateral nucleus.

Unfortunately, the material available does not allow a detailed localization of the long latency response seen in the dorsal part of the tracks through the ventrolateral nucleus. In Fig. 9 C and D, however, a track is shown with a lesion made at the depth where the response in that track changed from the long latency type to the response with shorter latency. From the figure it is evident that the response with long latency dorsally to the lesion was not derived from the ventrolateral nucleus. The track seems to have passed in its dorsal part through a pool of cells which may belong either to the nucleus ventralis anterior or the nucleus centralis lateralis.

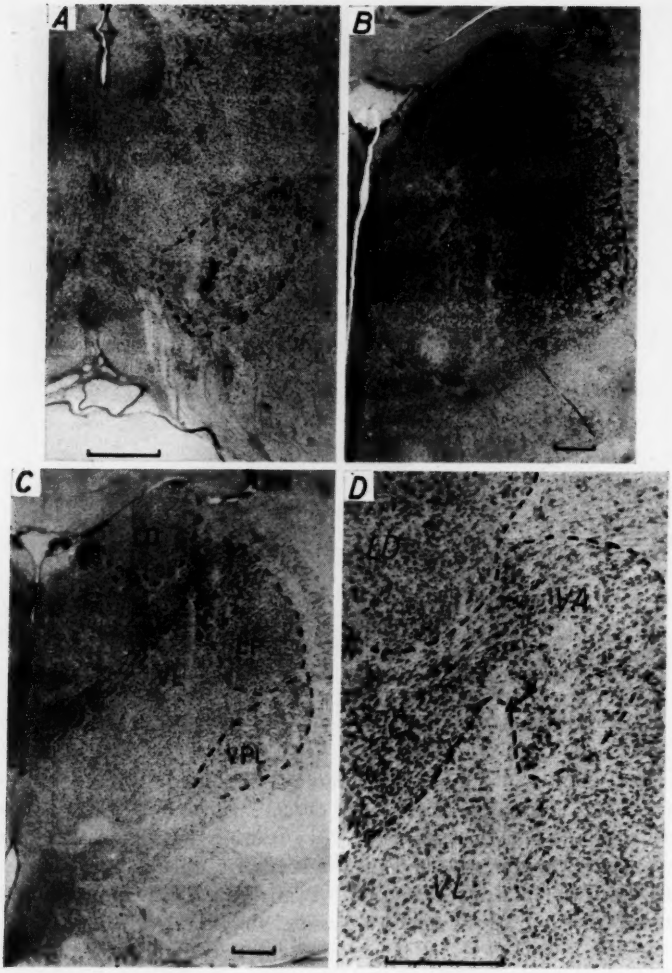

Fig. 9. A. Frontal section through the mesencephalon. The red nucleus is seen within the broken line. An electrolytic lesion is pointed at by the arrow.

B. Frontal section through the thalamus. An electrolytic lesion in the ventrolateral nucleus is seen at the arrow.

C and D. Different magnifications of a section through the thalamus. An electrolytic lesion (arrow) is situated at the dorsal border of the ventrolateral nucleus. Response of the long latency type was seen within about 2 mm dorsally to the lesion.

AV, nucleus anterior ventralis. CL, nucleus centralis lateralis. LD, nucleus lateralis dorsalis. LP, nucleus lateralis posterior. VA, nucleus ventralis anterior. VL, nucleus ventralis lateralis. VPL, nucleus ventralis posterolateralis. Scale 1 mm.

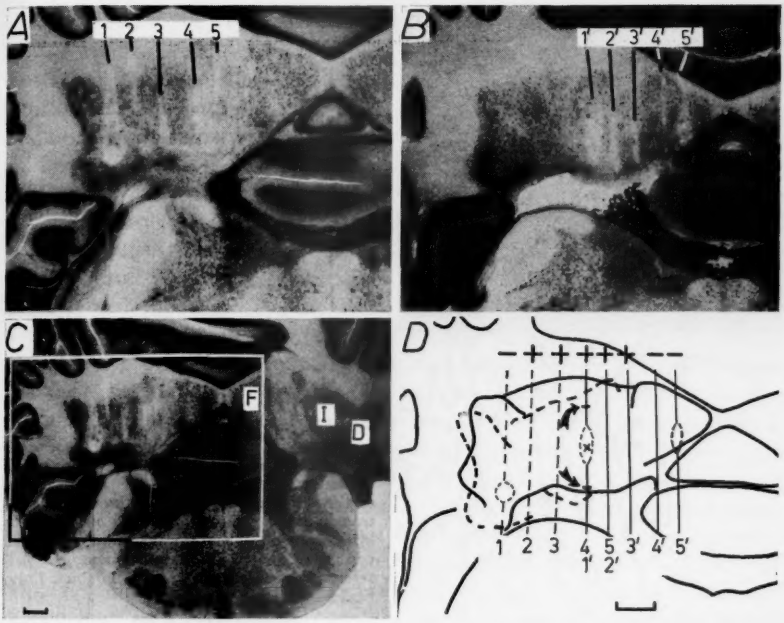

Fig. 10. Frontal section showing stimulating electrode tracks through the cerebellar nuclei. In A track 1 passes down into the dentate nucleus while the other tracks traverse the interposite nucleus. In B tracks 1', 2' and 3' pass through the interposite nucleus while tracks 4' and 5' enter the fastigial nucleus. C is a low power magnification of the same section as A. D is a composite diagram of A (nuclear borders drawn in broken lines) and B (nuclear borders drawn in full). Plus symbols indicate tracks in which electrical stimulation evoked a response in the red nucleus and in the ventrolateral nucleus. F, nucleus fastigii. I, nucleus interpositus. D, nucleus dentatus. Scale 1 mm.

III. *Localization of stimulating points*

These experiments were aimed at localizing the area within the cerebellar nuclei which, when electrically stimulated, activated the cells in the red nucleus and in the ventrolateral nucleus of the thalamus. A microelectrode was kept stationary either in the red nucleus or in the ventrolateral nucleus. The cerebellar nuclei were then investigated with mediolateral or rostrocaudal rows of stimulating electrode tracks. Fig. 10 and 11 are illustrations of such experiments. Fig. 10 C shows a low power magnification of a histological section through the cerebellar nuclei. A higher magnification of the framed area is seen in Fig. 10 A. Tracks 1, 2, 3, 4 and 5 are passing through the lateral part of the cerebellar nuclear complex. A lesion made by electrocoagulation is seen at the bottom of track 1. It is placed in the dentate nucleus. In Fig. 10 B is shown a similar section from another experiment where tracks 1', 2', 3', 4' and 5' enter the medial part of the nuclear complex. Electrocoagulations

Fig. 11. Sagittal section through the cerebellum about 4 mm laterally to the midline. The interposite nucleus with five stimulating electrode tracks is seen. Below is a diagram of the same section. Thick lines along the tracks indicate part of tracks effective in evoking a response in the red nucleus. The center of the electrocoagulation was used as a reference when measuring in the thick lines. Scale 1 mm.

are seen placed in the interposite (track 1') and the fastigial (track 5') nuclei. Fig. 10 D is a composite diagram of A (borders of the nuclei and tracks drawn in full) and B (borders and tracks drawn in broken lines). Electrical stimulation in the tracks with plus symbols above gave responses in the red nucleus and in the ventrolateral nucleus. In the dorsoventral direction stimulation was effective within the arrows in track 4/1'. This distance was determined with the center of the electrocoagulation (cross) in track 1' as a reference. It appears from Fig. 10 that only tracks passing through the interposite nucleus evoked a response in the red nucleus and in the investigated part of the thalamus. Tracks through the dentate and the fastigial nuclei were always negative in this respect. These observations were confirmed without exception in all experiments. An interesting observation was made in the experiments of the type illustrated in Fig. 11. In these rostrocaudal rows of tracks it was always noticed that the more caudally the stimulating electrode was situated within the interposite nucleus, the higher the stimulating intensity had to be in order to give a response of a certain amplitude. In fact, stimuli applied to the most caudal parts of the nucleus were rather ineffective. It is also evident from Fig. 11 that stimulation of the brachium conjunctivum rostrally and ventrally to the cerebellar nuclei was effective in evoking the focal potentials in the red nucleus and in the ventrolateral nucleus.

Discussion

The results presented seem to prove that stimulation of the interposite nucleus in the cerebellum activates the cells in the red nucleus and in the ventrolateral nucleus of the thalamus. This is in agreement with the findings of JANSEN and JANSEN (1955) that the bulk of the fibres from the interposite nucleus end in the red nucleus. This view was also supported by POMPEIANO (1958) who showed that motor effects elicited by electrical stimulation of the cerebellar cortex were abolished by destruction of the interposite nucleus or the red nucleus.

JANSEN and JANSEN also found that some fibres from the interposite nucleus, together with the majority of the dentate fibres, passed beyond the red nucleus. These fibres were thought to have thalamic connections. The present investigation has proved this view to be true with regard to the interposite fibres. On the other hand, it failed to show any connections between the dentate nucleus and the thalamus. From JANSEN and BRODAL (1958) it seems evident, however, that experiments directly demonstrating a relationship between the ventrolateral nucleus of the thalamus and the dentate nucleus are still lacking. The present findings do not support the view that such a relationship exists. It can, however, not be excluded that the dentate nucleus may have connections in other thalamic nuclei. Nor can it be excluded that the dentate nucleus impinges upon the cells in the ventrolateral nucleus (and maybe also in the red nucleus) in a way which is not elucidated by the technique used in this investigation.

An interesting detail in the work by POMPEIANO was that the lesion in the interposite nucleus had to be situated in the rostromedial part of the nucleus in order to abolish the motor effects observed. Experiments of the type shown in Fig. 11 in the present investigation seem to support this observation in that electrical stimulation is more effectively evoking the rubral response if carried out rostrally in the interposite nucleus.

The distance between the interposite nucleus and the red nucleus as measured on a histological section (shrinkage accounted for) is approximately 9 mm. The mean latency to the maximum of the initial positivity in the presynaptic spike recorded in the red nucleus is 0.49 msec. Adopting the interpretation of BROOKS and ECCLES (1947) this point signals the arrival of the presynaptic volley to the red nucleus. The conduction velocity may therefore be estimated to be about 18 m/sec. In the ventrolateral nucleus the corresponding latency is 0.94 msec and the total distance between the cerebellum and the thalamus is about 17 mm. The conduction velocity computed from these figures is also about 18 m/sec. As the difference in latency between the thalamic response and that recorded from the red nucleus is only 0.4 msec there seems to be no time for a synaptic delay in the path. The uniform conduction velocity computed from measurements made both caudally and rostrally to the red nucleus also suggests that the path from the cerebellum to the thalamus is a direct one and not interrupted by a synapse in the red nucleus.

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Oxygen Consumption and Sodium Reabsorption in the Kidney

By

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Abstract

LASSEN, N. A., O. MUNCK and J. H. THAYSEN. *Oxygen consumption and sodium reabsorption in the kidney.* Acta physiol. scand. 1961. *51.* 371—384. — Renal oxygen consumption can be divided into a small but constant basal oxygen uptake and a variable suprabasal or functional oxygen uptake. The basal oxygen consumption is defined as the oxygen uptake of the non-filtering and hence non-reabsorbing kidney. It was measured during marked hypotension and was found to amount to 1 μmole of O_2 per gram kidney per minute. The suprabasal or functional oxygen consumption is defined as the total oxygen uptake minus the basal. It was measured on normally functioning kidneys and was found to correlate very closely with the quantity of sodium reabsorbed. For each mol of oxygen consumed in excess of basal requirements 28 sodium equivalents were reabsorbed. The hypothesis, that sodium reabsorption represents the main renal work is forwarded. It provides a simple explanation for the various classical observations on renal oxygen uptake and is in agreement with data on other electrolyte transporting tissues.

In the isolated frog skin it was found by ZERAHN (1956 and 1958) that there was a basal or resting oxygen consumption, when the medium on the outside of the skin was sodium-free. When sodium was added to the medium, the skin started to transport sodium, and a functional oxygen uptake was now superimposed on the basal. This suprabasal or net oxygen uptake was dominated by and proportional to active sodium transport.

There is good evidence that about 99 per cent of the filtered volume in the mammalian kidney is reabsorbed in the tubules by a primary active inward transport of sodium, anions and water following passively due to the electrostatic and osmotic forces thus created. On a molar basis sodium reabsorption represents the overwhelming fraction of all tubular transport processes. Therefore, following the suggestion by USSING *et al.* in a recent review (1960), the present study was undertaken with the aim of investigating, whether a correlation between sodium reabsorption and oxygen uptake could also be demonstrated in the intact mammalian kidney. The results, which have been previously communicated briefly by THAYSEN, LASSEN and MUNCK (1959, 1960), are here presented and discussed in full detail.

Methods

Two different types of experiments were included in the study:

A. *Measurement of the oxygen uptake of the non-filtering kidney*

Four mongrel dogs were used. They were anesthetized with nembutal and throughout the experiment oxygen was administered at a rate of 1—2 l per min through a tracheal tube. The animals were heparinized. The abdomen was opened through a median incision and suitably large catheters tied into both ureters, one femoral artery and the right renal vein. Renal blood flow was measured by the free outflow method and the mean arterial blood pressure determined with a mercury manometer. Blood pressure was reduced to about half of its initial value by bleeding. When renal blood flow had been observed to remain stable for about 5 min samples of arterial and renal venous blood were drawn for determination of oxygen and plasma inulin. The stability of the renal blood flow was controlled after removal of the blood samples. By repeated bleedings blood pressure and renal blood flow were reduced stepwise. At each level the observations were repeated.

B. *Measurement of the oxygen uptake and sodium reabsorption of the filtering kidney*

Ten mongrel dogs were used. The preparation of the animals was the same as described above, except that sampling of renal venous blood in these non-heparinized dogs was carried out with a thin catheter introduced from the external jugular vein and guided manually into the left renal vein. A priming injection followed by a sustained infusion of paraaminohippurate (PAH) and inulin in isotonic saline was commenced in order to maintain arterial plasma concentrations of PAH and inulin at about 5—8 and 50 mg/100 ml, respectively. In exp. no. 9 and 10, urea was added to the infusate in order to secure an adequate urine flow. Two to three hours elapsed between the start of the infusion and the beginning of the measurements. In dogs no. 1 to 8 no attempts were made to interfere with renal blood flow. In dogs no. 9 and 10 the renal venous pressure was maintained at about 50—60 mm Hg by constriction of the renal vein throughout the study. 3—7 observation periods, each of 20—30 min duration, were obtained per dog. Diuresis was measured and the urine analyzed for PAH, inulin and sodium. In the middle of each period blood was drawn simultaneously from the femoral artery and the renal vein and examined for hematocrit, oxygen content, oxygen saturation, plasma inulin, plasma PAH and plasma sodium.

The correct position of the catheters was controlled at autopsy, and the kidneys

Table I. The basal oxygen consumption of the intact canine kidney

Dog	Observation period no.	Mean arterial blood pressure	Blood flow (ml/g/min)	(A—R) O_2 (μmoles/ml)	Renal oxygen uptake (μmoles/g/min)
A	1	58	1.44	0.72	1.04
	2	45	0.86	0.99	0.85
	3	30	0.35	2.29	0.80
B	1	65	1.06	1.04	1.10
	2	67	1.03	0.78	0.80
	3	60	1.06	1.03	1.09
	4	39	0.53	1.82	0.96
C	1	52	1.06	1.09	1.16
	2	37	0.57	1.64	0.93
D	1	47	0.62	1.81	1.12
	2	43	0.38	2.11	0.80
	3	44	0.52	1.63	0.85

Mean = 0.96 $\simeq$ 1.0
S. E. of mean = 0.04

were weighed after removal of capsule, pelvic fat and large vessels. In exp. 9 and 10 the experimental kidney was somewhat swollen, and the weight of the other kidney was used for the calculations.

Analytical methods and calculations

Blood oxygen content was measured manometrically as indicated by VAN SLYKE and NEILL (1924). The analyses were completed within 30 min after sampling, and the values obtained were compared to the results of simultaneous duplicate, photoelectric measurements of the oxygen saturation. Only the manometric analyses were used in the calculations. Inulin in plasma and urine was measured according to the method of ALVING, RUBIN and MILLER (1939). PAH was determined by the method of BRUN (1951). Sodium was measured by internal standard flame photometry. The hematocrit was measured by centrifugation. No correction was made for plasma trapping.

Renal plasma flow (RPF) in ml/g/min was calculated for each dog as the mean value of the plasma flows in the individual periods. Similar calculations were carried out for the hematocrit (H), the arterial-renal venous oxygen difference $(A - R)O_2$ in μmole/ml, the glomerular filtration rate (GFR) in ml/g/min, the plasma sodium concentration (P_{Na}) in μeq/ml, and for the urinary excretion rate of sodium ($U_{Na} \times V$) in μeq/g/min.

From these values the following parameters were calculated:

$$\text{Filtration fraction (FF)} = \frac{\text{GFR}}{\text{RPF}}$$

$$\text{Sodium reabsorption (Na reabs.)} = \text{GFR} \times P_{Na} - U_{Na} \times V \ (\mu\text{eq/g/min})$$

$$\text{Total renal oxygen uptake } (QO_2\text{total}) = \frac{\text{RPF}}{1 - H} \times (A - R)O_2 \ (\mu\text{mole/g/min})$$

Table II. Studies of renal sodium transport and oxygen metabolism in ten dogs. In dogs no. 1—8 no attempt was made to interfere with blood flow. In no. 9 and 10 the renal venous pressure was elevated to 50—60 mm Hg

Dog no.	No. of periods	RPF ml/g/min	H	RBF ml/g/min	(A—R) O_2 μmoles/ml	GFR ml/g/min	FF	Plasma sodium μeq/ml	Na filtr.	Na excret.	Na reabs.	QO_2 total	QO_2 net	Na/net O_2 ratio
									μeq/g/min			μmoles/g/min		
1	4	1.89	0.41	3.20	1.26	0.66	0.35	139	91.7	0.4	91.3	4.0	3.0	30
2	7	3.92	0.40	6.53	1.06	1.13	0.29	156	176.3	6.9	172.5	3.8	5.9	29
3	3	3.26	0.51	6.65	1.29	1.28	0.39	153	195.8	3.7	192.1	8.6	7.6	25
4	6	2.63	0.48	5.06	1.03	0.82	0.31	140	114.8	1.3	113.5	5.2	4.2	27
5	6	3.88	0.42	6.69	0.88	0.87	0.22	148	128.8	2.8	126.0	5.9	4.9	26
6	5	3.43	0.54	7.46	0.74	0.88	0.26	140	123.2	0.8	122.4	5.5	4.5	27
7	3	2.33	0.46	4.31	0.90	0.70	0.30	144	100.8	0.2	100.6	3.9	2.9	35
8	3	2.91	0.23	3.78	1.21	0.66	0.23	159	104.9	1.8	103.1	4.6	3.6	29
9	4	0.42	0.38	0.68	3.02	0.14	0.33	171	23.9	1.2	22.7	2.1	1.1	21
10	4	1.35	0.45	2.46	1.34	0.51	0.38	150	76.5	0.6	75.9	3.3	2.3	33
												Mean =		28
												S. D. =		4

Net renal oxygen uptake (QO_2net) = QO_2total — oxygen uptake of the non-filtering kidney.

Na/net O_2 ratio = number of sodium equivalents reabsorbed per mole oxygen = $= \frac{\text{Na reabs.}}{QO_2 \text{ net}}$

Experimental accuracy

The coefficient of variation in the determination of average net or suprabasal oxygen uptake was calculated to 13 per cent and that in the determination of sodium reabsorption to 4 per cent. These calculations are based on in average 4.5 observations of each parameter in the individual experiment.

Results

A. *The oxygen uptake of the non-filtering kidney*

Table I gives the values for mean arterial blood pressure, renal blood flow, arterial-renal venous oxygen difference and renal oxygen uptake. As demonstrated graphically in Fig. 1 (part A) the kidney studied during severe hypotension was found to react to a further reduction in blood flow with an increase in the a.v. oxygen difference, whereas the oxygen uptake remained fairly constant. Since no measurable a.v. difference for inulin could be detected in these experiments, we have interpreted the values for renal oxygen uptake as indicating the oxygen uptake of the non filtering kidney. It was found to average 1.0 μmole/g/min.

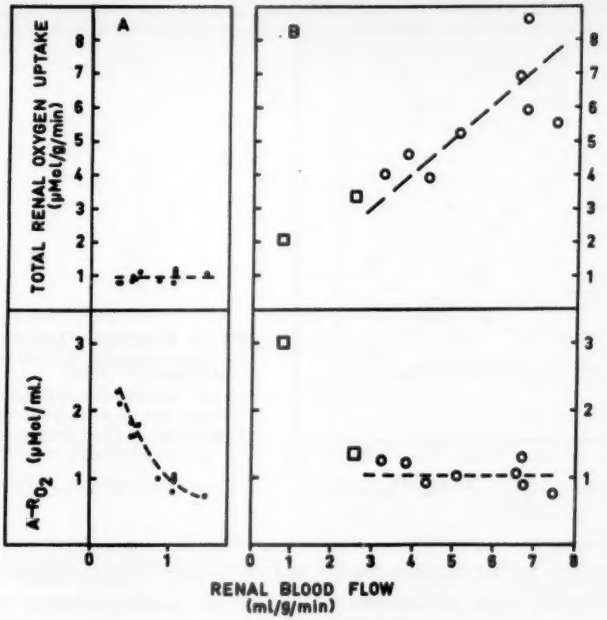

Fig. 1. A. Renal oxygen uptake of the non-filtering kidney in four dogs. Each point represents one observation period.
B. Renal oxygen uptake of the filtering kidney in ten dogs. Each point represents the average of 3—7 observation periods. In the experiments indicated by O no attempt was made to interfere with renal blood flow. In the experiments indicated by □ the renal vein was constricted.

B. *The oxygen uptake of the filtering kidney*

Table II gives the observations made in the dogs with normally functioning kidneys. The data have been analysed in some detail and the results of this analysis are presented in the following paragraphs.

The relationship between blood flow and total oxygen uptake

Fig. 1 (part B) shows that the a.v. oxygen difference remained fairly constant at about 1 μmole/ml in the 8 dogs with normal renal venous pressure despite variations in blood flow from 3.2 to 7.5 ml/g/min. In these experiments the total renal oxygen uptake consequently showed a rough correlation to renal blood flow. In the two experiments where renal venous pressure was increased, the a.v. oxygen difference was rather high and thus the total oxygen uptake was not reduced quite in proportion to the reduction in blood flow. Hence the observed rough correlation between blood flow and oxygen uptake may be upset by a marked increase in renal venous pressure.

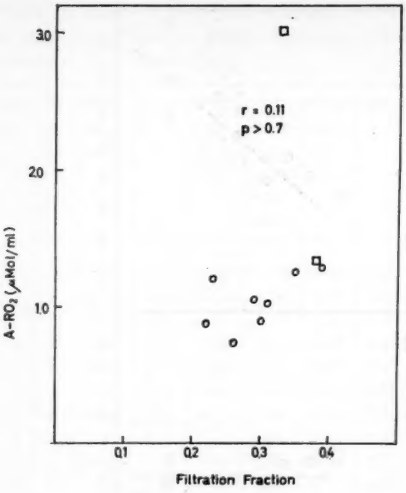

Fig. 2. Relationship between renal arteriovenous oxygen difference and filtration fraction.
In the experiments indicated by O no attempt was made to interfere with renal blood flow. In the experiments indicated by □ the renal vein was constricted.

The relationship between filtration fraction and arteriovenous oxygen difference

Fig. 2 shows that no significant correlation existed between these two parameters.

The relationship between filtration rate and total oxygen uptake

There was a highly significant correlation between the filtration rate and the total renal oxygen uptake. As seen in Fig. 3 the data suggest a linear relationship, and the calculated regression line indicates that 5.7 μmoles of oxygen are consumed per ml of filtrate formed. The intercept on the Y-axis represents the oxygen consumption of the kidney when no filtration takes place and was found to be 0.6 μmole/g/min (± 0.4). This value is somewhat lower than the value determined during haemorrhagic hypotension but the difference is not statistically significant.

The relationship between sodium reabsorption and total oxygen uptake

The correlation between these two parameters was equally good as the above described (Fig. 3). The coefficient of correlation between sodium reabsorption and total oxygen uptake was a little higher ($r = 0.98$) than between filtration rate and total oxygen uptake ($r = 0.97$), although the difference is not significant. It is not surprising that these two correlations are so similar since, in the present experiments, the filtration rate and the sodium reabsorption were highly intercorrelated due to the small variations in the plasma sodium concentration and in the excreted fraction of filtered sodium.

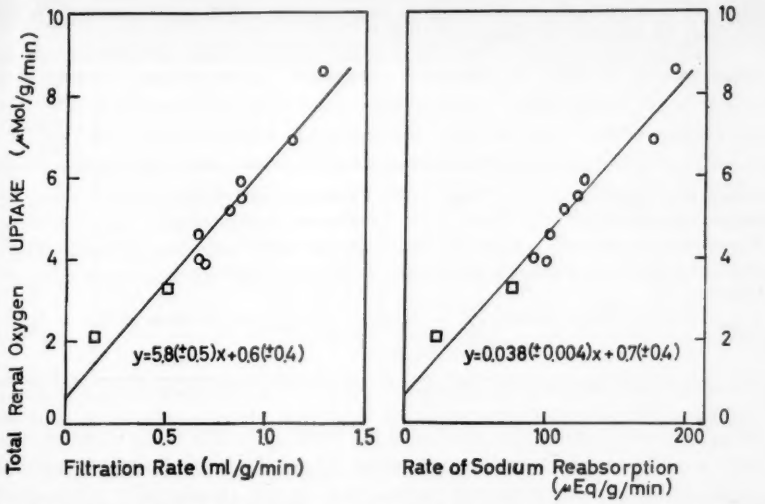

Fig. 3. Relationship between total renal oxygen uptake and filtration rate, and between total oxygen uptake and sodium reabsorption.
In the experiments indicated by O no attempt was made to interfere with renal blood flow. In the experiments indicated by □ the renal vein was constricted.

The ratio between sodium reabsorption and net oxygen uptake

The functional or net oxygen uptake was calculated by subtracting from the total oxygen uptake (in μmoles/g/min) the oxygen uptake of 1 μmole/g/min which was found in the non-filtering kidney. The ratio between sodium reabsorption and net oxygen uptake, the Na/net O_2 ratio, was on an average 28 with a standard deviation of 4, *i. e.* this value varied but little from dog to dog. The ratio denotes that 28 sodium equivalents were reabsorbed per mole of oxygen consumed.

Discussion

The basal oxygen uptake of the kidney

The definition of the basal or resting oxygen uptake of the kidney depends upon what we consider to be the major work process of the tubular epithelium, and its determination requires interference with normal physiological conditions, because the kidney is a normally never resting gland. Since the work of the tubular cells is to modify the glomerular filtrate into final urine, mainly by reabsorption of the filtrate, we have defined the basal oxygen uptake of the kidney as the oxygen uptake when no filtration and hence no reabsorption takes place. We have determined this basal metabolism in the artificial situ-

Table III. The basal oxygen consumption in various tissues (recalculated in μmoles of O_2 per g wet tissue; for references see Ussing et al. 1960)

Organ	Species	Reference	Basal oxygen uptake (μmoles/g/min)
Submaxillary gland	Dog	Terroux et al. (1958)	1.2
Submaxillary gland	—	Barcroft (1916)	1.0
Parotid gland	—	Ohara (1951)	1.4
Pancreatic gland	—	Still et al. (1933)	1.3
Kidney	—	Present Study	1.0
Kidney	Man (acute renal failure)	Munck (1958)	1.0

ation of severe hypotension produced by bleeding, and a fairly constant value over a wide range of variation in renal blood flow was found. Despite the unphysiological conditions this basal renal oxygen uptake of 1 μmole/g/min comes very close to the oxygen uptake in different non-stimulated exocrine glands and to the oxygen uptake of the anuric human kidney (Table III).

The finding of an intercept of about 0.6 μmole/g/min for the regression of total oxygen consumption on filtration rate or sodium reabsorption further supports the contention, that the kidney has a small, but constant basal oxygen uptake. The basal oxygen consumption obtained from these regressions is of the same order of magnitude as that obtained in the studies of the non-filtering kidney, but it is less exactly determined.

The basal oxygen uptake is probably used for preservation of the cellular structure and for extrusion of sodium leaking into the tubular cells over the inner (blood side) membrane. The small variations in the basal oxygen uptake from experiment to experiment suggest that the sodium leak is constant and relatively small.

The functional or net oxygen uptake of the kidney

As this value was determined by subtracting a constant from the total oxygen uptake, the net oxygen uptake has exactly the same highly significant and linear correlation to filtration rate and sodium reabsorption as the total oxygen uptake. When using the net oxygen uptake values the intercept on the y-axis practically disappears, demonstrating a direct proportionality between net oxygen uptake and filtration rate and between net oxygen uptake and sodium reabsorption. This suggests that either water reabsorption or sodium reabsorption is the dominant oxygen requiring work of the tubular epithelium. No other tubular transport process or synthesizing activity can be expected to parallel the filtered volume to such an extent. Moreover, on a molar basis

all such tubular processes are negligible as compared to water and sodium transport. The present experiments do not permit any conclusion as to whether water or sodium reabsorption requires the oxidative energy, since the two are highly intercorrelated. However, according to modern concepts reabsorption of filtrate takes place by a primary active reabsorption of sodium ions.

The following hypothesis regarding the renal oxidative metabolism is thus formulated: The total renal oxygen uptake comprises a relatively small and constant basal oxygen uptake of 1.0 μmole/g/min and a variable suprabasal or functional uptake which is utilized for sodium reabsorption at a rate of 1/28 mole O_2 per equivalent of Na reabsorbed. In quantitative terms this may be expressed by equation 1 (all values per g kidney per min):

$$QO_2 \text{ total} = (A - R)O_2 \times RBF = QO_2\text{basal} + QO_2\text{net} = \\ = 1.0 + 1/28 \text{ Na reabs} \qquad (1)$$

In the next paragraphs this hypothesis will be analyzed on the basis of various observations in the literature regarding renal oxygen consumption.

The proportionality between oxygen uptake and blood flow

In most organs a moderate reduction in blood flow leads to an increase in arteriovenous oxygen difference, so that the oxygen uptake remains unchanged. The kidney does not follow this pattern. It is evident from numerous observations — including the present series — that the renal a.v. oxygen difference most often is relatively independent of blood flow except at very low flows, so that there is rough proportionality between oxygen uptake and blood flow (HAYMANN and SCHMIDT (1928), GLASER *et al.* (1932), VAN SLYKE *et al.* (1934), LEVY *et al.* (1937, 1938), LEVY and BLALOCK (1938), KRAMER and WINTON (1939), DOLE *et al.* (1946), BRADLEY and HALPERIN (1948), BUCHT *et al.* (1949), CARGILL and HICKAM (1949)).

This peculiar behaviour of renal oxidative metabolism has caused much speculation. It has been suggested that the renal parenchyma was unable to extract oxygen from the blood below a certain critical limit. This theory was rejected by DOLE *et al.* (1946), who showed that the a.v. oxygen difference did not change at low arterial oxygen saturations.

The present hypothesis readily explains the relation between blood flow and oxygen uptake, as glomerular filtration and also tubular sodium reabsorption usually decrease in proportion to a decrease in blood flow. Thus the difference between the oxidative metabolism of the kidney and that of other organs is not due to a metabolic peculiarity of renal cells, but it is a mere consequence of the unique functional organization of the kidney: The amount of work to be carried out by the tissue is to a large extent determined by blood flow. When this functional pattern is abolished by marked hypotension stopping the filtration, the kidney — like any other organ — extracts more oxygen per ml of blood in response to a further reduction of

Table IV. Ratio of active electrolyte transport to net oxygen consumption in various tissues

Tissue	Ratio	Reference
Canine kidney	28	Present Study
Human kidney	30	Calculated from data of CARGILL & HICKAM and CLARK and BARKER
Submaxillary gland.....	16—25	Calculated from data of TERROUX, SEKELJ and BURGEN
Isolated frog skin.....	16—20	ZERAHN

perfusion and maintains a constant oxygen uptake until a very low rate of blood flow is reached (cf. Fig. 1).

In contrast with the above cited two groups of investigators have found that the renal oxygen uptake is independent of renal blood flow. CRUICKSHANK and TAKEUCHI (1925) observed in cats that an increase in the renal venous pressure produced a decrease in blood flow with an increase in a.v. oxygen difference. In experiments on dogs where blood flow was measured with a bubble-flowmeter connected to the renal vein GRUPP *et al.* (1958) found that the a.v. oxygen difference was dependent on the blood flow. In these two reports no data are given for filtration fraction or filtration rate, but the experimental conditions are suggestive of a high filtration fraction, so that also these results may well be in accordance with the concept that oxygen uptake is dependent on sodium reabsorption. This is discussed in more detail in the following.

The narrow arteriovenous difference and its variations

CLAUDE BERNARD (1858) first described the high oxygen contents of renal venous blood, an observation which suggests an ample supply relative to demand. In the terms of the present hypothesis the narrow a.v. difference $(A - R)O_2$ is the result of the fact, that only about 20 percent of the plasma flowing to the kidney is filtered under usual conditions; that the oxygen cost of sodium reabsorption is only 1/28 mol of O_2 per equivalent of sodium reabsorbed, and that the basal oxygen uptake is sufficiently small as to cause only a negligible oxygen extraction at the normal rate of perfusion.

The relative constancy of $(A - R)O_2$ when blood flow (RBF) is being reduced moderately has been commented on already. But the present theory even permits to make exact quantitative predictions of $(A - R)O_2$ in any state of function since equation (1) can be solved for $(A - R)O_2$ to yield equation (2) when inserting the previously defined symbols:

$$(A - R)O_2 \text{ in } \mu\text{mol/ml} = \frac{QO_2 \text{ total}}{RBF} = \frac{1.0}{RBF} + \frac{1}{28}(1 - H)FF \times P_{Na}(1 - E_{Na}) \quad (2)$$

When RBF is very small then $(A - R)O_2$ will increase quite markedly even when the sodium reabsorption per ml of blood ($(1 - H)FF \times P_{Na}(1 - E_{Na})$) is unchanged. Our experiment no. 10 illustrates this phenomenon which upsets the gross correlation between QO_2 total and RBF quite markedly. Further, equation (2) explains the large $(A - R)O_2$ in incompensated heart disease, since in this condition RBF is low and FF high (MERRILL 1946). The very narrow $(A - R)O_2$ found during albumin infusion also fits into equation (2) since here RBF is high and FF low (BARKER *et al.* 1949). Finally, equation (2) permits to predict that no strict correlation can be expected to exist between $(A - R)O_2$ and FF unless all the other variables are kept reasonably constant. This lack of significant correlation between $(A - R)O_2$ and FF was observed by DOLE *et al.* (1946) and also in the present series (cf. Fig. 2). On this basis DOLE *et al.* rejected the possibility that the renal oxygen uptake should be dependent on the amount of filtrate to be handled by the tubular tissue. Their conclusion was probably wrong, as the present data show that there was a highly significant correlation between *net* oxygen uptake and sodium reabsorption even though $(A - R)O_2$ and FF varied independently. DOLE *et al.* did not consider the possibility of a basal oxygen uptake.

Miscellaneous observations on renal oxygen uptake. CARGILL and HICKHAM (1949) and CLARK and BARKER (1951) have published data on the oxygen uptake of the normal human kidney. Their average value from a total of 28 observations can be calculated to be 3.1 μmoles/g/min when assuming a renal weight of 300 g. From their data for the glomerular filtration rates the average sodium reabsorption in the same subjects comes at 60 μeq/g/min assuming a plasma sodium concentration of 142 μeq/ml and a negligible sodium excretion. From these data in man a $Na/netO_2$ ratio of about 30 is obtained, *i. e.* almost the same value as in the present experiments on dogs.

BUCHT *et al.* (1949) and CLARK and BARKER (1951) studied the effect of variations of renal PAH excretion on renal oxygen uptake in man. They found no significant correlation between the two parameters. This is in agreement with the present hypothesis since the maximal PAH transport measured in eq/min is very small compared to sodium reabsorption. Similarly, variations in renal glucose or aminoacid reabsorption cannot be expected to influence renal oxygen uptake measurably. Moreover, the possibility of a coupling between sodium transport and one or more of the other transport mechanisms cannot be excluded, a possibility implying that these other transport mechanisms may not *per se* require oxidative energy.

A very interesting observation was made by CARGILL and HICKAM in studies of the renal oxygen uptake in chronic renal diseases. In these diseased kidneys a direct proportionality was found between the reduction in total oxygen

uptake and in glomerular filtration rate. This is in agreement with the present hypothesis if it is assumed that the weight of metabolically active renal tissue and hence basal oxygen uptake was also reduced roughly in proportion to the reduction in glomerular filtration rate.

The renal efficiency. The minimal energy required to form urine from plasma ultrafiltrate can be calculated by applying simple thermodynamic considerations to the quantitatively important substances (VON ROHRER 1901). This minimal energy constitutes only about 1 per cent of the total energy released by the oxidative metabolism in the kidney (BORSOOK and WINEGARDEN 1931). This value of about one per cent for the efficiency of renal work is surprisingly low compared to that of the muscles, and moreover it was shown to vary considerably (EGGLETON, PAPPENHEIMER and WINTON 1940). In view of the present hypothesis thermodynamic calculations of the minimal renal work are irrelevant since they disregard the utilization of oxygen for *isotonic* sodium reabsorption. Since the present theory of renal work is based on the stoechiometric considerations formulated by ZERAHN for the frog skin the concept of a renal efficiency in the usual thermodynamic sense of the word cannot be used.

The hypothesis that the net (suprabasal) oxygen consumption of the kidney is dominated by and stoechiometrically related to active tubular sodium reabsorption has been shown to be consistent with a series of classical observations on the oxidative metabolism of the kidney. It is of interest in this context that the oxygen consumption of exocrine glands other than the kidney is apparently also best explained in terms of active electrolyte transport (USSING *et al.* 1960).

When not secreting the exocrine glands have a relatively constant basal oxygen uptake (Table III). When stimulated to discharge a functional oxygen consumption is superimposed on the basal. The suprabasal or functional oxygen consumption must be largely used for the processes underlying glandular discharge, since the synthesis of enzymes and other specific substances proceeds at a relatively constant rate under all conditions. In the glands as in the kidney the functional oxygen consumption has been correlated with the thermodynamic work involved in the formation of a secretory product deviating in ionic composition from the original plasma. A minimal "glandular efficiency" was found in this way.

Such calculations, however, disregard the oxygen cost of the isotonic active outward transport of electrolyte (water following passively), which represents the basis of the secretory process according to modern concepts of glandular function. If one calculates from data in the literature the ratio between active electrolyte transport and net oxygen consumption this ratio, the stoechiometric efficiency, is found to vary from 16 to 25 (Table IV).

In 1958 TERROUX *et al.* showed that the arteriovenous oxygen difference in the non-stimulated salivary gland was 3—4 μmol/ml. When the gland was stimulated to secrete the a.v. oxygen difference decreased considerably and

approached that found in the kidney. With the knowledge that glandular blood flow varies roughly in proportion to secretory rate, and that the "secretion fraction", like the filtration fraction of the kidneys, is about 0.25, the observations of TERROUX *et al.* can be explained on the basis of the concepts developed in the present paper: At high rates of blood perfusion the basal oxygen demands of the gland and of the kidney only contribute insignificantly to the a.v. oxygen difference. In both gland and kidney net oxygen demands dominate the a.v. oxygen difference in this situation. Net oxygen demand per ml of perfusing blood is determined by active outward (gland) or inward (kidney) transport of comparable quantities of electrolytes originating from secretion or filtration fractions of the same order of magnitude.

The ratio between active electrolyte transport and net oxygen consumption of the kidney is compared to the ratios found in other intact organs and in the isolated frog skin in Table IV. Whether the difference between the epithelial membranes (isolated or intact) are significant is difficult to state, and depends to some extent on the accuracy with which the basal oxygen consumption of the membranes is determined. The larger the basal uptake is in comparison to total uptake during maximal transport, the larger error may arise from an inaccurate determination of basal metabolism. For these reasons it is as yet impossible to state whether the stoechiometric efficiency of the electrolyte transporting mechanism varies from one epithelial membrane to the other.

Addendum in proof: Recently ULRIK LASSEN and J. H. THAYSEN (Biochim. et Biophys. Acta. *47:* 616, 1961) demonstrated an intimate correlation between the active sodium extrusion from and the suprabasal oxygen consumption of renal cortical slices from rabbits. A Na/net O_2 ratio of about 25 was found. KRAMER and DEETJEN (Excerpta Medica, Congr. Ser. *29:* 50, 1960; Klin. Wschr. *38:* 680; 1960: Arch. ges. Physiol. *271:* 782, 1960) have obtained similar results on the intact canine kidney as those presented in the present paper.

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The Contribution of the Distribution Factor to the A-a Po_2 Difference (A Correction)

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In a recent publication on the alveolo-arterial gas exchange (1960) we found that the arterial oxygen deficit in rest breathing air was 0.36 vol% of which the part due to true venous admixture was found to be 0.08 vol%. Since the part of the *A—a* Po_2 difference due to incomplete diffusion equilibrium can be considered to be negligible the remainder of the arterial oxygen deficit 0.36 vol%—0.08 vol% = 0.28 vol% in air will be due to the distribution factor.

The effect of the distribution factor on the *A—a* Po_2 difference must at different levels of alveolar Po_2 be proportional to the change of slope of the O_2-dissociation curve, *i. e.* to the second derivative of this curve. The contribution of the distribution factor to the arterial oxygen deficit in the experiments with 12 % O_2 was, therefore, estimated to be about 20 times larger than during air breathing *i. e.* sufficient to cover the whole arterial O_2 deficit at 12 % O_2. By this estimation we unfortunately overlooked the fact that the same difference in ventilation/perfusion ratio produces a smaller Po_2 difference between the relatively hypo- and hyperventilated parts of the lung at 12 % O_2 than at 21 % O_2. This is caused by the fact that, due to the shape of the O_2-dissociation curve, a given relative hypoventilation will diminish the oxygen uptake in the hypoventilated part of the lung more at 12 % O_2 than at 21 % O_2. Correspondingly the oxygen uptake in the hyperventilated part of the lung will increase more at 12 % O_2 than at 21 % O_2.

We have calculated the alveolar Po_2 and Pco_2 differences between relatively hypoventilated and hyperventilated parts of the lung at 21 % O_2 and at 12 % O_2 in two cases of uneven distribution. It was assumed that the ventilation in the hypoventilated part of the lung was reduced to 50 % or to 67 % of the mean,

and, in the hyperventilated part, increased to 150 % or 133 % while the circulation remained constant. It was tentatively assumed that the alveolar gas tensions were equal to the end pulmonary capillary gas tensions. Further it was presumed that no regulatory changes in ventilation and circulation took place at 12 % O_2. The respective alveolar Pco_2 and Po_2 values were determined as those that allowed the gas exchange from the inspired air to the alveoli to equal the gas exchange from the alveoli to the lung capillaries in each of the two parts of the lung, while at the same time the total gas exchange remained constant. The calculations showed that the Po_2 difference between the hypoventilated and the hyperventilated parts of the lung in both cases was 3.5 to 4 times smaller at 12 % O_2 than at 21 % O_2.

The decrease in O_2 saturation, ΔS %, of the arterial blood that occurs when equal amounts of blood from the relatively hypoventilated and the relatively hyperventilated parts of the lung are mixed can be expressed as:

$$\Delta S\ \% = \tfrac{1}{2}(\Delta P)^2 + \left(\frac{d^2S}{d\,P^2}\right)\bar{P}$$

where ΔP is the difference in alveolar Po_2 between the two parts of the lung, and $\left(\frac{d^2S}{d\,P^2}\right)\bar{P}$ i the second derivative of the O_2 dissociation curve at mean alveolar Po_2.

Since ΔP was found to be 3.5 to 4 times smaller at 12 % O_2 than at 21 % O_2, whereas the second derivative of the oxygen dissociation curve (curve III, Fig. 3, ASMUSSEN and NIELSEN 1960) is 22 times larger, it follows that at 12 % O_2 ΔS % is only about 1.5 times larger than at 21 % O_2. As the part of the arterial O_2 deficit which was due to the distribution factor was found to be 0.28 vol% at rest breathing air, this factor will increase to about 0.42 vol% at 12 % O_2. True venous admixture (0.08 vol%) and uneven distribution (0.42 vol%) will, therefore, contribute 0.5 vol% or 25 % of the total arterial deficit which at 12 % O_2 was found to be 2 vol%. The remaining part (75 %) of the arterial O_2 deficit must be assumed to be due to failing diffusion. The lung diffusion constants for O_2 calculated under the erroneous assumption that at 12 % O_2 the main part of the arterial oxygen deficit was due to uneven distribution are consequently too high.

By the calculation it was tentatively assumed that there was no diffusion gradient from alveolar air to end capillary blood. The result of the calculation however, showed that a diffusion gradient actually exists at 12 % O_2. A precise calculation of the contributions of failing diffusion and uneven distribution to the A—$a\,Po_2$ difference at low oxygen can, therefore, hardly be performed.

Reference

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